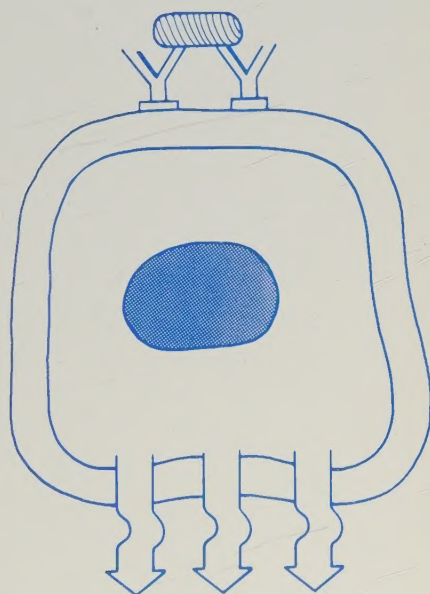


ANTIGEN-INDUCED BRONCHIAL ANAPHYLAXIS IN ACTIVELY SENSITIZED SPRAGUE DAWLEY RATS

CHARACTERIZATION OF THE BRONCHIAL RESPONSE
IN RELATION TO SERUM ANTIBODY
LEVELS, MEDIATORS AND DRUGS



Magnus Dahlbäck

Lund 1986



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ANTIGEN-INDUCED BRONCHIAL ANAPHYLAXIS IS ACTIVELY SUSCEPTIBLE TO SERUM DILUTION

CHARACTERIZATION OF THE BRONCHIAL RESPONSE
TO ANTIGEN IN SERUM DILUTION
AND THE EFFECTS OF ANTIGEN AND DILUTION

By
J. H. HARRIS

To Eva, Anna and Anders

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PREFACE

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I. M. Dahlbäck.
Antigen-induced bronchial anaphylaxis in actively sensitized SD rats. Effects of immunization and provocation doses of antigen and of pretreatment with DSCG, theophylline, terbutaline and a new anti-allergic xanthine derivative, D4026. *Allergy* 1981, 36: 583-595.
- II. M. Dahlbäck, R. Pauwels, H. Bazin & H. Bergstrand.
Relation between bronchial reactivity to antigen in vivo and serum IgE and IgG_{2a} antibody levels in rats. *Allergy* 1982, 37: 569-580.
- III. M. Dahlbäck, H. Bergstrand, R. Pauwels & H. Bazin.
The non-specific enhancement of allergy. III. Precipitation of bronchial anaphylactic reactivity in primed rats by injection of alum or B. pertussis vaccine: Relation of response capacity to IgE and IgG_{2a} antibody levels. *Allergy* 1983, 38:261-271.
- IV. M. Dahlbäck, H. Bergstrand & L. Sörenby.
Bronchial anaphylaxis in actively sensitized Sprague Dawley rats: Studies on mediators involved. *Acta pharmacol. et toxicol.* 1984, 55: 6-17.
- V. M. Dahlbäck, H. Bergstrand & R. Brattsand.
Antigen-induced bronchial anaphylaxis in actively sensitized SD rats: Effects of glucocorticoid treatment. *Allergy* (in press).
- VI. M. Dahlbäck.
Antigen-induced bronchial anaphylaxis in actively sensitized SD rats: Effects of local treatment with anti-asthmatic drugs. Submitted to *Allergy*.

ABBREVIATIONS

AA	Arachidonic acid
ACA	Active cutaneous anaphylaxis
ACh	Acetylcholine
Ae	Aerosol
1-B	1-Benzylimidazole
BAR	Bronchial anaphylactic reaction
BN	Brown Norway
Bp	Bordetella pertussis
BRAD	Bradykinin
BUD	Budesonide
CY	Cyclophosphamide
DEX	Dexamethasone
DNP	Dinitrophenyl
DSCG	Disodium cromoglycate
FCA	Freund's complete adjuvant
FRC	Functional residual capacity
GCS	Glucocorticosteroid(s)
HA	Haemagglutinin
HD	High dose of antigen challenge
HL	Hooded Lister
Hp	Haemophilus pertussis
5-HT	5-Hydroxytryptamine (serotonin)
i.g.	Intragastrally
Ig	Immunoglobulin
i.m.	Intramuscular(ly)
i.n.	Intranasal(ly)
INDO	Indomethacin
i.p.	Intraperitoneal(ly)
i.t.	Intratracheal(ly)
ITP	Intratracheal pressure
i.v.	Intravenous(ly)
LD	Low dose of antigen challenge
LT	Leukotriene(s)
Nb	Nippostrongylus brasiliensis
NDGA	Nordihydroguaiaretic acid
OA	Ovalbumin
PAF-acether	Platelet-activating factor (L- α -lecithin, β -acetyl- γ -0-alkyl)

PCA	Passive cutaneous anaphylaxis
PG	Prostaglandin(s)
PLA	Passive lung anaphylaxis
PU	Prast unit(s)
s.c.	Subcutaneous(ly)
SD	Sprague Dawley
SPF	Specific pathogen-free
SRS-A	Slow reacting substance of anaphylaxis
TERB	Terbutaline
THEO	Theophylline
TNP	Trinitrophenyl
TPP	Transpulmonary pressure
TxA ₂	Thromboxane A ₂

Fig. refers to Figures in the Papers

FIGURE refers to Figures in the Summary

INTRODUCTION

Almost 300 years ago Samlon referred to asthma as a disease characterized by "difficult respiration, sometimes with and sometimes without fever, sometimes with a noise and sometimes without, arising from an obstruction of bronchia and cells of the lungs" (Lessof 1984). Today the definition of asthma is somewhat more stringent (Scadding 1985) although it varies with authors. Apart from reversible bronchoconstriction, the characteristics of asthma also encompass oedema formation, airway inflammation, hypersecretion from mucous glands and sputum retention (Casale & Marom 1983, Clark & Godfrey 1983, Weiss et al 1985).

Bronchial asthma occurs in both children and adults. It has been calculated that in the United States and in Australia 7% of children and 5% of adults suffer from the disease (McFadden 1984). In Great Britain the highest estimate of current prevalence of asthma for adults was 5.4% (Clark & Godfrey 1983). In Sweden, asthmatic symptoms associated with respiratory infection have been found in 10-15% of pre-school children and the prevalence of asthma symptoms in school children and adults was 2.5%. Generally twice as many males as females develop asthma following puberty (Foucard 1983). Recent studies estimate that at least 40% of asthmatics develop their disease by the age of 10 in all Western countries, whereas only 33% develop their asthma after 45 years of age (Lessof 1984).

Allergy in the respiratory tract is a common feature of asthma especially in young people. The correlation between asthma and skin test reactivity is higher in younger than in older subjects (McFadden 1984). In allergic asthma (often referred to as extrinsic), an attack is elicited by an external allergen (e.g. pollen, animal epithelia or house dust mites) to which the patient is allergic. In occupational asthma, which may also be immunologically mediated, the triggering factor can be metal salts, industrial chemicals such as isocyanates or dusts from wood, flour or cotton.

In allergic asthma an IgE antibody response is thought to play a

central role (Johansson & Foucard 1978, Cockcroft et al 1979). The IgE antibodies become fixed to high affinity receptors in the membranes of mast cells and basophils. On renewed exposure to allergen, crosslinking of the IgE receptors will occur, which initiates the transduction of a signal to the interior of the cell. This ultimately leads to basophil/mast cell degranulation and the release of preformed and newly synthesized mediators. Homocytotropic antibodies of the IgG₄ type have been demonstrated in man, but it is still unknown whether this type of antibody contributes to the pathogenesis of asthma (Nakagawa 1983, Stanworth 1983, Perelmutter 1984). The mast cell and basophil mediators involved in anaphylactic reactions in man include histamine, arachidonic acid metabolites (leukotrienes, prostaglandins and thromboxanes) and possibly platelet-activating factor as well as various chemotactic factors and proteolytic enzymes (Marom & Casale 1983, O'Flaherty & Wykle 1983, Schwartz & Austen 1984).

When an allergic individual is exposed to a relevant allergen an immediate bronchoconstriction usually occurs. This immediate bronchospasm is thought to be induced by mediators released from mast cells which act either directly or through the nervous system. The dominant nervous control system of the lung in man is the cholinergic system, a stimulation of which among other things leads to constriction of the bronchial smooth muscle. The adrenergic system may via β_2 -receptors induce relaxation of bronchial smooth muscle and may via α -receptors contribute to a constrictory response (Richardson 1979, Nadel 1980). Moreover, the so-called nonadrenergic, noncholinergic inhibitory system, which acts by an as yet undefined mediator has been suggested to be involved in regulation of airway smooth muscle tone (Barnes 1984, Richardson 1985).

The immediate asthmatic reaction is often followed by a second, so-called late phase reaction, beginning 4-8 h and persisting up to 24 h after challenge (Kaliner & Lemanske 1984, Kay et al 1984). This late phase reaction is characterized by cellular infiltration, initially consisting of neutrophils. Later on eosinophils may contribute considerably to this cellular infiltrate. These observations have led to the concept that inflam-

matory cells may be responsible for the late phase reaction and/or the ensuing non-specific bronchial hyperreactivity (Nadel & Holtzman 1984, Nadel 1984, Boushey & Holtzman 1985).

This non-specific hyperreactivity of the airways is an important characteristic of bronchial asthma (Cockcroft et al 1977, Hargreave & Dolovich 1984, Hogg et al 1985). It is manifested as an exaggerated response to exogenous non-allergic stimuli such as various smooth muscle spasmogens (e.g. histamine and methacholine) or to other physiological stimuli such as irritants. Irritants like SO_2 , ozone, nitrogen dioxide and formaldehyde usually have an immediate bronchoconstrictory effect but in contrast to extrinsic asthma no immunological causal factors can be identified (Nadel & Sheppard 1985). Asthmatic individuals react more vigorously to such constricting agents than normal individuals. This type of hyperreactivity is often increased in atopics after an allergen provocation or after virus infections (Hargreave 1983).

Amongst the most effective drugs used in the treatment of asthma are β_2 -stimulating agents such as terbutaline (TERB), salbutamol etc. These agents cause smooth muscle relaxation but may also act by a number of other mechanisms to induce relief from symptoms (e.g. by inhibition of mediator release from mast cells, by reduction in microvascular permeability and by increase in mucociliary clearance) (Morley 1984).

Xanthine derivatives (e.g. theophylline, THEO) are another class of effective anti-asthmatic drugs which probably exert their action by several different mechanisms (Svedmyr 1984, Holgate et al 1984, Andersson & Persson 1985).

Yet another group of drugs used in asthma is glucocorticoids, (GCS), which exhibit anti-inflammatory and anti-allergic actions. These agents generally show beneficial effects also in patients with severe asthma (Clark et al 1982, Harding 1984, Morris 1985 a,b, Schleimer 1985, Tse and Bernstein 1985).

Disodium cromoglycate (DSCG) is an anti-asthmatic drug which shows only prophylactic properties, i.e. it is active when given

before an allergen exposure but it does not regularly induce symptomatic relief. Pepys (1981) showed that DSCG could block the development of both the immediate and the late asthmatic reaction. DSCG is also known to be effective in controlling exercise-induced asthma in a substantial proportion of patients with this reaction. However, DSCG cannot be given orally with retained activity; furthermore, not all patients respond to the drug (Buckle 1985, Bernstein 1985).

After the introduction of DSCG numerous models were employed in an effort to find drugs that could mimic the action of DSCG preferably after oral administration. These models were chosen with reference to the concept that DSCG acts by inhibiting mediator release from mast cells. Models used were antigen-induced histamine release from chopped human and guinea-pig lungs, and passive cutaneous anaphylaxis (PCA) in rats. However, none of the models could convincingly select new drugs with a reliable activity in the clinical situation (Church 1978, Stokes & Morley 1981). This led to the search for new animal models which could better select drugs effective in the treatment of allergy and asthma.

Diseases in the respiratory tract, that are due to a reagin-mediated type of allergy do not normally occur in animals, with the exception of dogs which may be sensitive to ragweed pollen (Patterson & Kelly 1974). However, it has been clearly demonstrated that after challenge with the pertinent antigen, immediate bronchospasms can be experimentally induced in appropriately sensitized rhesus monkeys (Patterson & Talbot 1972), sheep (Wanner et al 1979), dogs (Patterson & Kelly 1974, Frey et al 1984), rabbits (Shampain et al 1982), guinea-pigs (Andersson 1980a), rats (Stotland & Share 1974a) and mice (McCaskill et al 1984). The immunological mechanism associated with the bronchial response to antigen provocation in these models is mainly considered to be a reagin-mediated (IgE) reaction; it is thus similar to that of human allergic asthma (Johansson & Foucard 1978). In addition it has recently also been reported that experimental allergic late bronchial responses can occur in sheep (Abraham et al 1983, rabbits (Shampain et al 1982, Larsen et al 1984) and guinea-pigs (Brattsand et al 1985a, Takishima et

al 1985). However, today there is no ideal small animal model which mimics the non-allergic stigmata of asthmatic subjects i.e. the non-specific airway hyperreactivity.

In 1958 Sanyal and West described anaphylactic shock in the albino rat. We know today that the antigen-induced bronchial anaphylactic reaction (BAR) in the rat shows many similarities to the immediate bronchoconstriction in human asthma, one being the immunological triggering mechanism. Moreover, drugs such as DSCG and GCS at least partly block the reaction in both man and rat (Church et al 1972). However, the mediators involved in BAR in the rat are not the same as those in man. In the rat 5-HT plays a central role as a mediator whereas in man (and in the guinea-pig) histamine seems to be a more important bronchoconstrictor. Other mediators involved in the BAR in rats seem to be acetylcholine (ACh) and various metabolites of arachidonic acid (AA). With respect to non-specific bronchial hyperreactivity, Brunet et al (1983) demonstrated, in an inbred strain of SD rats, the existence of an increased reactivity to 5-HT. The expression of this bronchial hyperreactivity was unrelated to the allergic state. Similarly it was recently shown that inbred rat strains differ in bronchial reactivity to 5-HT independently of their IgE-producing capacity (Pauwels et al 1983a). On the other hand, Ahlstedt et al (1983) reported that immunized rats respond less vigorously to exogenous 5-HT than non-immunized rats.

Many authors have studied the BAR in rats, using different rat strains and different conditions in inducing and eliciting the response. Some characteristics of these models are shown in Tables 1 to 3. The strains generally used are Sprague Dawley, Wistar and Hooded Lister (HL). Rats have been sensitized to the nematode *Nippostrongylus brasiliensis* (Nb) which in itself possesses adjuvant activity for IgE in rats (Church et al 1972, Church 1975a,b), by ovalbumin (OA) injections without adjuvant (Smedegård et al 1984), or by injections of OA together with adjuvants such as *Bordetella pertussis* (Bp) vaccine with or without alum (Stotland & Share 1974a,b, Farmer et al 1975, Church & Miller 1978, Carswell & Oliver 1978a,b, Piechuta et al 1979, Holme & Piechuta 1981, Brunet et al 1983), or with alum

alone (Church & Miller 1978, Diamond et al 1978). Animals may also under certain circumstances be sensitized by aerosolized antigen (Van Houte & Johnson 1972, Piechuta et al 1979, Ahlstedt et al 1983). Passive sensitisation with high titre IgE antibody sera has also been utilized (Church 1975b, Farmer et al 1975, Piechuta et al 1979). Challenge has mostly been performed by the intravenous route or through exposure to aerosolized antigen. The response has been measured either as increase in intratracheal pressure (ITP), tracheal flow reduction, transpulmonary pressure restriction (TPP), changes in compliance and/or resistance (C_{dyn}/R_1), or as changes in respiratory pattern. However, in none of the studies recorded has there been a convincing correlation between BAR and circulating specific IgE antibody level (Table 2).

We were interested in characterizing the BAR model in some detail with respect to its immunological parameters with the hope of giving a better understanding of some of the contradictory results so far reported. We were also interested in trying to find conditions where BAR correlates to serum IgE or IgG_{2a} antibody levels. We therefore decided to study the temporal development of the capacity of the rats to elicit a BAR in order to identify the optimum conditions for immunization and the optimum antigen challenge dose.

It was hoped that the studies described in this thesis might provide some answers to the following questions. Could the variable results obtained with different drugs used to inhibit BAR depend on the different immunological conditions used? Could a clarification of the immunological parameters lead to a model which might be used to better characterize clinically used drugs (among other things, with respect to possible differences between local and systemic treatment) and to select new anti-asthmatic and/or anti-inflammatory drugs for therapy in man?

TABLE 1

A SUMMARY OF CHARACTERISTICS OF BAR IN RATS

	Strain	Immunization		
		Antigen	Adjuvant	Route
Sanyal & West (1958)	Albino	Horse serum, egg white, hog mucin, OA	Hp	i.p./i.p.
van Houte & Johnson (1972)	SD	OA (1%)	Bp	Ae/i.p.
Church et al (1972) Church (1975a,b)	Wistar	Nb		s.c.
Church & Miller (1978)	Wistar	OA (100µg)	Al(OH) ₃ (1mg)	i.p./i.p.
Stotland & Share (1974a,b)	SD	OA (1mg)	Al(OH) ₃ (200mg), Bp	s.c./s.c. i.p.
Farmer et al (1975)	SD Wistar	OA (5mg/kg) Passive	Bp, Nb	i.m./i.p. i.v.
Carswell & Oliver (1978a,b)	Hooded Lister	DNP-OA (10µg) Booster DNP-OA (1µg)	Bp	i.p./i.p.
Diamond et al (1978)	SD	OA (2x50µg)	Al(OH) ₃	i.p./i.p.
Piechuta et al (1979)	SD, Long- Evans, Wistar, Fischer344	OA (1mg) 1% (5')	Al(OH) ₃ (200mg) Bp, Nb	s.c./s.c. i.p. Ae (not effective)
Holme & Piechuta (1981)	SD (inbred)	OA (1mg)	Al(OH) ₃ (200mg) Bp	s.c./s.c. i.p.
Piechuta & Holme (1982)	---	---	---	---
Brunet et al (1983)	--- Fischer344	---	---	---
Ahlstedt et al (1983)	BNxWi/Fu	OA (0.01-1%) x 1 or 2w		Ae
Smedegård et al (1984)	---	OA (.1µg) x 2w		s.c.
Ufkes et al (1983) (1984)	BN	TNP-OVA (13µg)	AlPO ₄	i.p.

TABLE 2

A SUMMARY OF CHARACTERISTICS OF BAR IN RATS

Provocation					
	Route	Time (days)	Dose	Type of response	Relation to IgE
Sanyal & West (1958)	i.v.	12-18	1ml	Visual shock	ND
van Houte & Johnson (1972)	ND	14	ND	PCA HA	ND
Church et al (1972)	i.v.	35	500 worm eq/kg	Tracheal flow, PCA, ACA	
Church (1975a,b)	"	"	"-	"-	BNC
Church & Miller (1978)	i.v.	14	.5-2mg	Tracheal flow PCA	ND
Stotland & Share (1974a,b)	i.v.	14	10mg	ITP (P%) PCA	BNC
Farmer et al (1975)		25		PCA, tracheal overflow,	ND
	i.v.	1	25mg/kg	PLA	
Carswell & Oliver (1978a,b)	i.t.	30	2mg DNP-OA 20mg/ml OA 10-20'	Respiratory pattern, PCA	BNC
	Ae i.n. i.g.				
Diamond et al (1978)	i.v.	12-15	10mg/kg	C _{dyn} /R ₁	ND
Piechuta et al (1979)	Ae	14-18	3%OA(1')	Respiratory pattern, PCA	BNC
Holme & Piechuta (1981)	"	"-	"-	"-	
Piechuta & Holme (1982)	"	"-	"-	"-	
Brunet et al (1983)	"	12-14	"-	"-	
Ahlstedt et al (1983)	Ae +i.v.	7,14 re- peat- edly	10mg/ml OA 10' + 5mg	TPP	BNC
Smedegård et al (1984)	"-	"-	"-	"-	
Ufkes et al (1983) (1984)	i.v.	12-21	.5 mg/kg	C _{dyn} /R ₁	BNC

ND = not determined
 BNC = BAR not correlated to reagin antibody titre

TABLE 3a

SUMMARY OF CHARACTERISTICS OF BAR IN RATS

	Effects of mediators-	Effects of drugs on BAR
Sanyal & West (1958)	5-HT + Histamine +	
Church et al (1972)		DEX ++ DSCG ++ Methysergide +++ Meclofenamate - DEX+Meclofenamate ++ Atropine - Mepyramine - Propranolol - Adrenalectomy -
Church (1975a)	Histamine - 5-HT +++ ACh +++ BRAD ++ SRS-A - PGF _{2α} -	Methysergide +++ (5-HT)* Atropine ++ (5-HT, ACh)* Mepyramine - Meclofenamate - Propranolol -- (BRAD)* DEX ++ (BRAD)*
Church & Miller (1978)		DEX ++
Stotland & Share (1974b)		Methysergide +++ Mepyramine + Burimamide - INDO - Mecamylamine - Atropine + VAGOTOMY - DSCG + DEX + DEX+DSCG +++ Isoproterenol ++ PROP --
Farmer et al (1975)		Mepyramine - Methysergide ++ FPL 55712 ++ Aspirine - Aprotinin - DSCG ++ VAGOTOMY -

+, low inhibition;
 ++, medium inhibition;
 +++, high inhibition;

-, no inhibition;
 --, potentiated reaction;
 *, mediator induced bronchial response.

TABLE 3b

SUMMARY OF CHARACTERISTICS OF BAR IN RATS

	Effects of mediators	Effects of drugs on BAR
Carswell & Oliver (1978a)		DSCG ++
Diamond (1978)	5-HT +++ Histamine - PGF ₂ - ACh ₂ - BRAD - Methacholine +++	Tiaramide ++ Methysergide +++
Piechuta et al (1979)		Epinephrine +++ DSCG +++ THEO +++ DEX +++
Piechuta & Holme (1982)	5-HT +++	Methysergide +++ FPL 55712 ++ NDGA ++ BW 755C ++
Brunet et al (1983)	Methacholine +++ 5-HT +++ LTD ₄ + Histamine+5-HT +++	INDO - (LTD ₄)*
Ahlstedt et al (1983)	5-HT +	INDO +++
Ufkes & Ottenhof (1984)		Prednisolone +++ FLP 55712 + Mepyramine - Ketotifen ++ DSCG ++

AIMS OF THE PRESENT INVESTIGATION

- to define optimum conditions for antigen-induced bronchial anaphylaxis in vivo in Sprague Dawley rats with respect to immunization and provocation doses of antigen and time of challenge.
- to describe the relation between bronchial reactivity in vivo and serum IgE and IgG_{2a} antibody levels.
- to define the kind of mediators involved in the bronchial anaphylactic response.
- to elucidate the clinical relevance of this model by examining the efficacy of different anti-asthmatic and/or anti-inflammatory drugs with various routes of administration.

MATERIALS AND METHODS

In general the materials and the methods used in the present examinations have been given in detail in each particular paper. Therefore, here I will only briefly summarize sources of animals and materials and methods used.

A Animals

Outbred male Sprague Dawley (SD) rats of SPF status were obtained from Møllegaards Breeding Center Ltd., Ejby, Skensved, Denmark. The breeding nucleus of the Brown Norway (BN) strain (source: Sloan-Kettering Institute via Wallenberg Laboratory, Lund) and the Fischer strain (source: Møllegaard Breeding Center Ltd., Ejby, Skensved, Denmark) were kindly provided by Prof. B. Källén, Lund. Both male and female rats of the inbred strains were used. The animals were reared under standard animal housing conditions; breeding was performed by strict brother-sister mating.

B Antigen

Ovalbumin (OA) grade III (Sigma Chem. Co.) was used as an antigen and the amount given either by i.p. or s.c. injection is specified in the enclosed papers (I-VI) or below.

C Adjuvant

The adjuvants used were 100 mg of a dried preparation of $\text{Al}(\text{OH})_3$ (F2200 Reheis, Chem. Co., US. obtained through AB Astra, Södertälje, Sweden), 10 mg of amorphous silica gel (Aerosil 200, Degussa, Frankfurt), 2 ml of Perthydral (a whooping cough vaccine produced by Institut Pasteur Production, Paris, containing 2.5×10^9 organisms of Bp and 1.25 mg $\text{Al}(\text{OH})_3$ per ml), or 0.2 ml of equal parts of an OA-solution and Freund's complete adjuvant (FCA, Difco Lab., Detroit, Michigan) appropriately emulsified (II, III). Some animals were injected with 35 or 50 mg/kg cyclophosphamide (CY, Sendoxan, Pharmacia, Uppsala, Sweden) (I).

D Immunization

The OA solution and the adjuvant were mixed at least one hour before being injected. Care was taken to obtain an evenly suspended adjuvant by shaking the mixing flask and inverting the syringe immediately before each injection. An immunization dose of 10 µg OA given together with 100 mg Al(OH)₃ was the most frequently used inoculum. In addition to the immunization procedure mentioned in Papers I-IV, rats were also immunized s.c. with 1 mg OA in 200 mg Al(OH)₃ or with OA given alone i.p. without an adjuvant.

E Respiratory measurements

The animals were anaesthetized at the indicated time after immunization. The trachea was cannulated and connected to a respiratory pump. The intratracheal pressure was measured through a side arm of the cannula connected to a pressure transducer. Arterial blood pressure was recorded via another pressure transducer. All recordings were made on a Grass polygraph.

F Antigen challenge

Animals were challenged i.v. through a catheter inserted in the external jugular vein. Based on the dose-response curves reported in Paper I, a low antigen dose (normally 0.3 mg OA or 0.5 mg to rats immunized with 100 µg OA in IV, V) was injected and the response recorded. Approximately 2 min later an additional high dose (5 mg OA) was often injected and the response again recorded. Occasionally, provocation was performed with the high challenge dose only. Anaesthetized animals were challenged by aerosol via a respiratory pump. The nebulized solution containing 1% OA in 3% glycerol saline was generated from a Bird nebulizer 890 at an air pressure of 0.2 MPa.

G Drugs

A number of drugs have been used to characterize the BAR. These drugs were used with the aim of reducing the reaction either by

interfering with the antigen-induced release of mediators from mast cells (Papers I, V, VI) or by interfering with the action of these mediators (Paper IV). Some agents were examined for direct agonist/mediator action (IV). The rationale for examining each particular drug, the handling of these drugs and the doses employed are given in the above mentioned papers.

H Drug administration

Acute drug administration was performed either i.v., i.p., i.t. or by aerosol at the indicated time before challenge (see Papers I-VI). During long-term treatment, dimaprit (a putative histamine H₂-agonist kindly synthesized by Dr. Jan Trofast, Draco) cimetidine (Tagamet^R, Apoteksbolaget), ketotifen (Sandoz, Basel) and other anti-asthmatic and anti-inflammatory drugs (sources specified in the appropriate paper) were administered by one i.p. injection daily 5 days a week for 3 weeks. The treatment was ended 3 to 7 days before test.

I Determination of serum levels of OA-IgE, OA-IgG_{2a} and total IgE

Blood samples were drawn under ether anaesthesia from each animal by retro-orbital bleeding or from the right jugular vein or carotid artery just before challenge at the indicated time (see II, III). During long-term treatment blood was taken before the start, at the end of treatment and/or before challenge. All antibody determinations were performed by radioimmunoassay techniques at Prof. R. Pauwel laboratory in Gent, Belgium.

Paper-radio-immuno-sorbent-test (PRIST) was used for quantitative analysis of total IgE antibodies (Pauwels et al 1977a) (see FIGURE 1). The principles of this test are as follows: An anti-IgE antibody containing serum was produced in goats by injection of purified myeloma-derived IgE (IR2 IgE). The anti-IgE-antibodies of this antiserum were absorbed to a Sepharose column to which purified myeloma IR162 IgE was coupled and the IgE class-specific antibodies were eluted with 0.1M glycine pH 2.8. By using another myeloma IgE for absorption than that used for immunization, anti-idiotypic antibodies were considered not

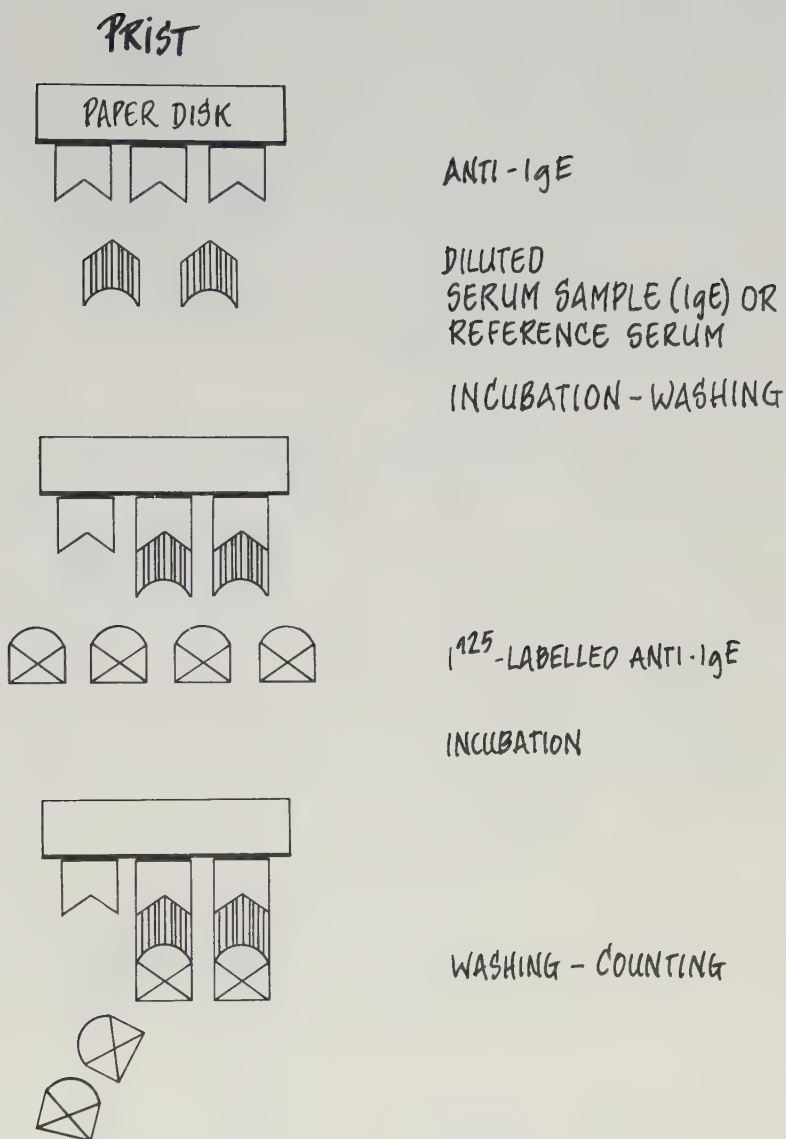


FIGURE 1

A schematic summary of the PRIST technique.

to be retained on the immunosorbent. The purified anti-IgE antibodies were linked to a paper disc in a polystyrene tube. Serum containing the unknown amount of IgE or a reference serum with a known amount of IgE, appropriately diluted, was added. The paper disc fixed anti-IgE bound the serum IgE. After incubation and washing, I^{125} -labelled anti-IgE was added. The labelled anti-IgE then in a sandwich manner bound to the serum IgE (see FIGURE 1). After incubation, the sample was washed, centrifuged, and the radioactivity remaining on the disc was counted in a gamma counter. The amount of IgE in a serum was deduced by comparison with a reference serum with a known IgE concentration and was expressed in ng/ml.

Paper-radio-allergo-sorbent-tests (PRAST) were used for quantitative determination of specific OA-IgE antibodies (Pauwels et al 1977b) (see FIGURE 2). The antigen (OA) was coupled to a paper disc in a polystyrene tube and the serum sample was then added. After incubation, the solid phase antigen-antibody complex was washed and the proteins not bound to the antigen were thus removed. I^{125} -labelled anti-IgE antibodies were then added to the tube. After incubation and washing, the radioactivity remaining on the solid phase was counted in a gamma counter. The amount of specific OA-IgE antibodies was deduced by comparison with a reference serum and expressed in arbitrarily defined PRAST Units/ml (PU/ml). Specific OA-IgG_{2a} antibodies were measured in a similar way as for OA-IgE utilizing an antiserum specific for IgG_{2a}, and the amount of specific OA-IgG_{2a} antibodies was expressed in PRAST units/ml (PU/ml) (Bazin and Pauwels 1982).

J Statistics

In series where a sufficient number of parallel experiments could be performed, the figures recorded for the bronchial anaphylactic response were found not to deviate from a normal distribution. Mean values and standard errors of the mean are used for graphical illustrations. The results of experiments evaluating inhibition of BAR in drug-treated versus vehicle-treated animals were statistically tested with Student's t-test or analysis of variance with or without linear contrast. Wil-

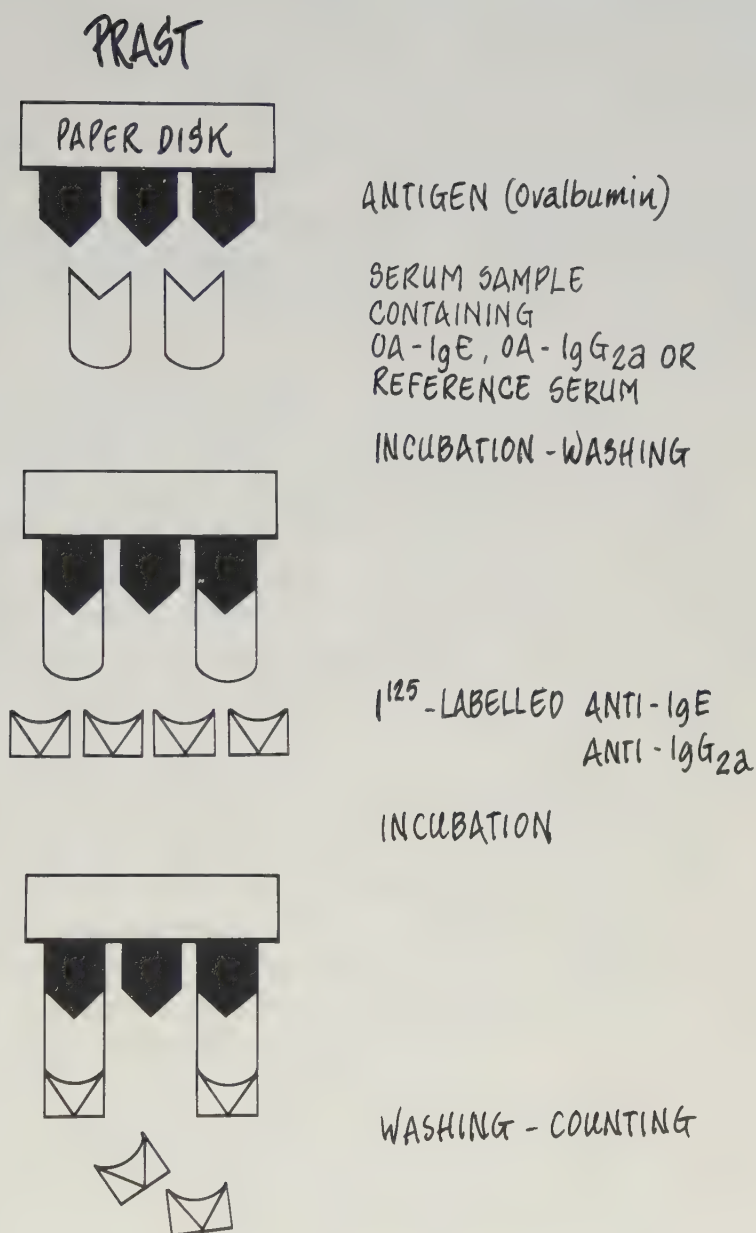


FIGURE 2

A schematic summary of the PRAST technique.

coxon's test was used for comparison of serum IgE and OA-IgE and OA-IgG_{2a} antibody levels, since these variables did not distribute normally.

RESULTS AND DISCUSSION

A Rationales for the choices of respiratory measurement technique, antigen, adjuvant, route of immunization, and strain of rats

a. Respiratory measurement

Respiration can be monitored in conscious rats by measuring either flow or pressure changes. This gives the observer an idea of the respiratory pattern. Piechuta et al (1979), Holme & Piechuta (1981) and Brunet et al (1983) studied respiratory responses in conscious rats and Carswell & Oliver (1978a,b) in anaesthetized rats, sensitized to OA when these animals were challenged with aerosolized antigen. The two groups focused on different aspects of the response pattern, the former on "apnea and/or dyspnea" and the latter on "expiratory notching", both parameters being comparatively rough estimates of the allergen-induced respiratory changes. The respiration of unanaesthetized animals with a more persistent state of lung disease can also be followed by forced oscillation (resistance) or compliance measurements (Murphy & Ulrich 1964, Swann et al 1965, Hiett 1974). These methods give the observer the opportunity to study the temporal development of the lung disease within the same animal, both in acute and chronic situations.

With anaesthetized animals many different methods have been utilized for characterizing allergen-induced changes in respiration. These methods include measurements of, e.g., changes in intratracheal pressure (ITP) (Stotland & Share 1974a), peak expiratory flow rates (PEFR), forced vital capacity (FVC) (Holroyde et al 1982), resistance (R_1), compliance (C_{dyn}) (Amdur & Mead 1958 and Andersson 1980) and closely related parameters such as transpulmonary pressure (TPP) (Smedegård et al 1984) and flow reduction (Church 1975a).

Studies in anaesthetized animals have their benefits in that it is possible to follow many physiological parameters at the same time, e.g. blood pressure, heart rate, and respiration. In conscious animals usually only one parameter can be studied at the same time. Moreover, the precision in the measurements of lung mechanics and in the delivery of drugs (e.g. i.v.) is often increased when anaesthetized animals are employed. However, the respiratory response to an antigen challenge may differ between anaesthetized and conscious animals (compare results of Carswell & Oliver 1978a and of Piechuta et al 1979). Such a difference may be the result of a disturbance in the sedated animals of the reflex mechanism that normally regulates the bronchial muscle via the parasympathetic vagal nerve.

In preliminary studies we found changes in ITP to be a reliable and reproducible parameter of the BAR in rats. The increase in ITP after antigen challenge is due mainly to an increase in resistance in the central airways (>2 mm diameter) (Macklem 1970). We also found that the change in ITP following antigen challenge of sensitized rats shows the same level of sensitivity as changes in R_1 , (results not shown).

Barbiturates have often been used to sedate animals in acute studies. These, however, - especially pentobarbitone - are long-lasting anaesthetics with dose-related depressant effects on respiration (Green 1979). By combining a hypnotic, metomidate (which depresses the respiratory rate but increases the amplitude) with pentobarbitone, it was possible to reduce the dose of pentobarbitone and still to reach a stable level of anaesthesia without any drop in the blood pressure during the experiment. Therefore, we decided to use ITP measurements in anaesthetized animals for examination of optimal conditions for induction of a BAR in relation to IgE and IgG_{2a} antibody syntheses.

b. Antigen

Agents with potent allergen-inducing capacity in man often consist of proteins with various molecular weights. However, the basis for their allergenicity in biochemical terms is not known (Tada 1975, King 1985). Pollen is one of the best studied groups of allergens. In an experimental study of mice and rats

immunized with timothy grass pollen, Nordvall et al (1982) demonstrated that the specificity of the IgE antibody response was similar to that in atopic humans. However, there is no study on the BAR in rats using pollen extracts as an antigen. A disadvantage in using pollen as an antigen is that the properties of pollen extracts may vary from batch to batch. Two antigens frequently used in animal experimental systems are OA and Nb (Hirshman & Downes 1985). OA is most commonly used because its immunogenicity is good, and it is obtainable in batches with reproducible properties.

c. Adjuvant

The immunological response of the rat in terms of IgE antibody production (and also in terms of BAR; see below) to a single injection of an antigen such as OA is normally low. However, it can be enhanced by including in the inoculum a suitable adjuvant. Adjuvants that potentiate the IgE antibody synthesis in rodents include *Ascaris*, Nb, Bp vaccine, alum and silica (Tada 1975, Mancino & Bevilacqua 1978). FCA is normally considered unfavourable for the stimulation of IgE production (Björkstén & Ahlstedt 1984, Ishizaka 1985). However, some authors report adjuvant activity for IgE with FCA (Bennich et al 1978). Both Bp vaccine or silica produces enhanced IgE responses which are generally transient in nature, while alum may induce more long-lasting responses which may also be enhanced by a second injection of the antigen (a booster).

Although there are still many unsolved questions concerning the regulation of reaginic antibody synthesis, the actions of the above-mentioned adjuvants may (partly) be explained within the following framework. The IgE biosynthesis is regulated by antigen non-specific but isotype-specific T-helper and T-suppressor cells through the action of different types of factors. Such different types of factors are for instance IgE B-cell generating factor, IgE-binding factors such as the IgE-potentiating and IgE-suppressive factors, the inducers of IgE-binding factors such as glycosylation inhibiting and glycosylation enhancing factors or their equivalents. These factors together act in either suppressing or enhancing the IgE antibody production (for reviews see Tada 1975, Katz 1978, 1980, 1984,

Ishizaka 1983, 1985). Alum and Bp on one hand and FCA on the other have been reported to enhance the production of factors leading to an increase or a decrease in the IgE synthesis (Ishizaka 1983, 1985). However, the mechanism of action of silica is less well known. Therefore we thought that $\text{Al}(\text{OH})_3$, silica and Bp could be useful tools in the efforts of developing a persisting IgE-mediated BAR capacity.

d. Route of immunization

The preferable route of immunization in a model of allergic asthma would seem to be by inhalation. Van Houte & Johnson (1972) examined rats immunized with a single exposure of aerosolized OA together with an i.p. injection of Bp. They did not use BAR measurements but found that these animals developed high PCA titres two weeks after immunization. Piechuta et al (1979) exposed animals to OA-aerosols repeatedly without detecting any development of BAR. Ahlstedt et al (1983) sensitized animals by repeated exposure to aerosolized OA without employing an adjuvant. However, when these animals were challenged with aerosolized OA only a weak BAR developed. Challenge by the i.v. route led to a more pronounced response. Ahlstedt & Björkstén (1983) showed that daily i.n. or p.o. administration to rats of OA alone did not result in any OA-IgE response, but when rats were given antigen s.c. a production of OA-IgE was detected. This was confirmed in another paper by the same authors (Ahlstedt et al 1985) where rats were immunized s.c., either in the neck or in the tail-root, or by aerosol. Rats immunized in the neck reacted markedly to i.v. challenge and less markedly to aerosol challenge one week after immunization, whereas animals immunized in the tail-root responded seven to eight weeks after repeated immunization. Sedgwick & Holt (1985) exposed BN rats repeatedly to an aerosol of OA and found increasing levels of OA-specific IgG in serum. However, the IgE antibody response was transient. This study provides evidence that IgE isotype specific suppressor T-cells may regionally control IgE responses in the lung to inhaled antigen. This may explain the normally low degree of sensitization to aerosolized antigen seen in the rat.

Jarrett (1978) found that neither the dose of antigen (OA) nor its route of administration (i.p., i.d. or p.o.) did essentially

influence the specific IgE antibody production in HL rats. Gerbrandy & Bienenstock (1976) determined the kinetic appearance of IgE-producing cells in various lymphoid tissues of mice following i.t., i.p. or s.c. immunization with tetanus toxoid and Bp. They found the highest degree of IgE(-antibody) producing cells in the bronchial lymph nodes after either i.p. or i.t. immunization, whereas the s.c. route led to lower levels of IgE-producing cells. As we were interested in immune responses related to the lung, we initially immunized rats either by i.t. instillation of OA or by aerosolized OA and challenged them by aerosol or i.v. No BAR was recorded under any circumstances. In these experiments alum was occasionally given as an adjuvant either locally or i.p.; again no BAR could be observed (data not shown).

Experiments were also performed in which OA was given i.p. as a single injection without an adjuvant. No BAR was seen at appropriate challenge. However, if sufficient amounts of OA were given daily five days a week for two weeks, a weak BAR was recorded at an ensuing challenge of the rats (I). These results suggested to us that an adjuvant was needed in order to prime the rats to develop a pronounced BAR. We also noticed that an adjuvant had to be given simultaneously with the antigen to exert its optimum effect at the primary immunization (III). An experiment was therefore performed in which rats were immunized with 1 mg OA together with 200 mg $\text{Al}(\text{OH})_3$ s.c. They were challenged i.v. with four cumulative doses of OA three weeks later. These rats responded only to the highest challenge dose (5 mg OA) (see FIGURE 3). With regard to these data and the report of Gerbrandy and Bienenstock (1976) mentioned above, we decided to immunize the animals by the i.p. route.

e. Strain of rats

Homocytotropic antibody production has frequently been studied in rats (Tada 1975, Ishizaka 1976, Bennich et al 1978, Jarrett 1978, Katz 1980, Bazin & Pauwels 1982). Initially, these antibodies were assayed by PCA technique, but after the identification of monoclonal plasmacytoma derived IgE in the rat, it was possible to develop radioimmunological techniques for determination of both total and antigen specific IgE (Bennich et al 1978, Bazin & Pauwels 1982).

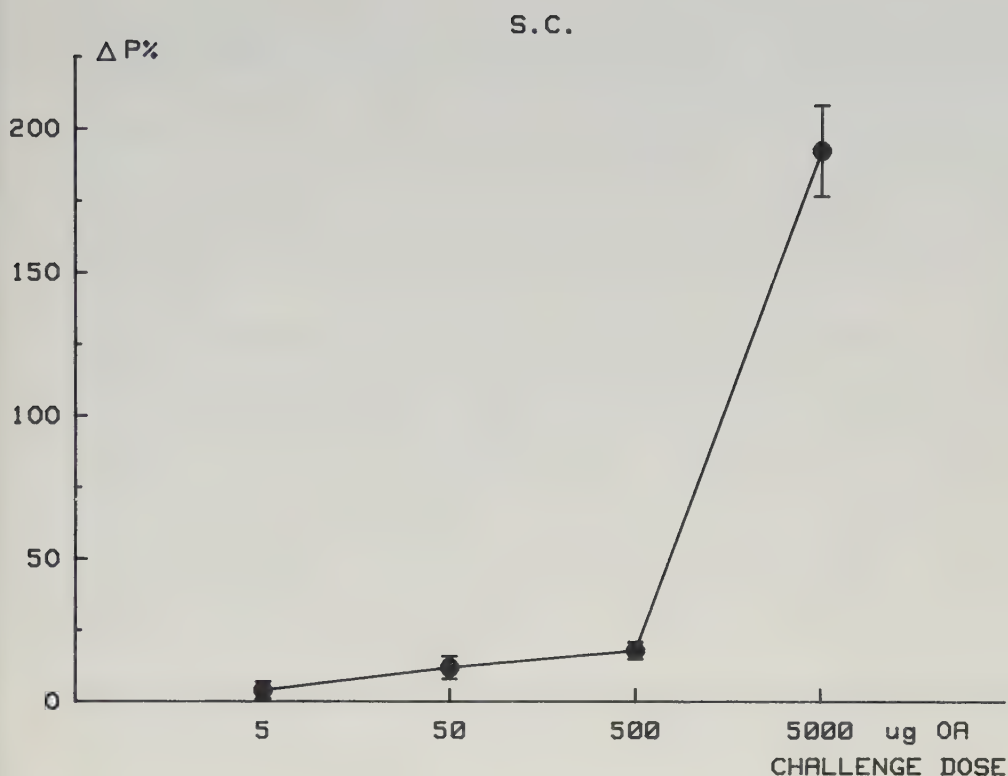


FIGURE 3

Effects on the BAR of increasing challenge doses

SD rats immunized with 1 mg OA and 200 mg $Al(OH)_3$ s.c. and challenged i.v. 25 days after sensitization with four cumulative doses of OA. Each point denotes the mean and SEM of BAR of 5 experimental animals.

The basal level of total IgE varies between rat strains. BN and HL rats normally have high levels of IgE, Wistar, Lewis and Fischer rats display medium to low levels, and SD rats show low levels of IgE (Bennich et al. 1978, Pauwels et al 1979a). Pauwels et al (1979a) showed a significant positive correlation between the total serum IgE before immunization and the specific IgE antibody production after immunization. Thus, they found that BN and HL rats with high basal levels of total IgE after immunization showed a high increase in specific IgE antibody

production, whereas Wistar rats, which have low to medium levels of IgE, showed low levels of specific IgE after sensitization.

In pilot experiments we immunized rats of different strains i.e. BN, Fischer and SD, with low doses of OA together with alum and examined the resulting degree of BAR. The SD strain gave the highest and the most reproducible BAR. This was unexpected since, as mentioned, the SD strain is considered a low IgE responder strain. However, the BAR can be thought to be influenced not only by specific IgE antibody levels but also by the degree of non-specific airway reactivity which varies with strain independent of IgE antibody producing capacity (Brunet et al 1983, Pauwels et al 1983a). Moreover, there are other studies showing differences between rat strains regarding more general pulmonary functions. For example, Boyd et al (1982) measured airway resistance and thoracic gas volume in male SD and Fischer rats and found that SD had a larger gas volume and less resistance than Fischer rats. Actively sensitized (Stotland & Share 1974a, Church 1975a, Carswell & Oliver 1978b, Church & Miller 1978, Piechuta et al 1979) as well as passively sensitised SD rats (Farmer et al 1975) have been used in BAR studies by other authors.

Bennich et al (1978) observed that animals bred and/or housed under conventional conditions had higher serum levels of IgE compared with animals kept under SPF conditions. This could be explained by the occurrence of different types of infections in conventionally bred animals. A highly increased synthesis of IgE is also seen in animals infected by endoparasites such as Nb (Jarrett 1978). To minimize environmental influence on the degree of IgE antibody synthesis it seemed preferable to use animals of SPF status with low total IgE antibody levels.

Male rats of the SD strain are larger than those of most other strains and consequently more suitable for lung-function studies (ITP). Therefore, we decided to use outbred male SD rats of SPF status, approximately two months old at a body weight of about 200 g. This is an optimal age for induction of an IgE antibody response (Pauwels et al 1979b, Bazin & Pauwels 1982).

We first immunized such rats by a single i.p. injection with low doses of OA together with different adjuvants e.g. alum, silica, and Perthydral and also injected OA together with FCA. We found that animals immunized with 10 μ g OA together with alum showed a strong and persistent capacity to mount a BAR when challenged two to six weeks later with a low dose of antigen (I). Such animals were also challenged by aerosolized OA delivered via a respiratory pump (10 mg/ml 10 to 20 min). No change in ITP was seen after the provocation with aerosolized OA despite the large BAR recorded after i.v. challenge (data not shown).

Taken together, these results indicate that the antigen at immunization should be given parenterally (e.g. i.p.) together with a suitable adjuvant and that the appropriately sensitized animals should be challenged i.v. to obtain a considerable degree of a reproducible BAR. Although antigen exposure by inhalation is the normal route in man, the rat seems to respond less efficiently to aerosolized antigen. The reason for this is not clear.

B. Characterization of the dose-response relationship for OA-sensitization and provocation with special reference to the temporal development of BAR capacity

Appropriately immunized animals challenged i.v. with a low provocation dose of antigen show a clearcut increase in their ITP. Fig. 1 in Paper I shows a typical recording from a SD rat immunized with 10 μ g OA in 100 mg alum which was challenged with a LD ($\Delta P\%$) and a HD ($\Delta P_{max}\%$) two weeks after immunization. The response to an i.v. challenge was fully developed within one to two minutes. Nonsensitized animals did not show such a response. In Paper I, Fig. 2 gives the results recorded for groups of animals, immunized with different doses of OA, which two weeks after sensitization were challenged with a single dose of antigen. Before the first increase in ITP starts to decay, an additional higher antigen dose may be given and the ITP may be recorded until the maximum pressure response is reached. To examine whether provocation with a low dose of antigen affects the response to an ensuing challenge with a high dose of antigen, animals immunized with 100 μ g or 1000 μ g OA were challenged

at two weeks in a cumulative way with the provocation doses used in Paper I Fig. 2. These animals exhibited the same degree of response to the high antigen dose as those given it as a single injection (data not shown). Thus, a low provocation dose of antigen given before a maximum challenge does not influence the response to this high provocation dose. This was found to be the case also in animals immunized with 10 μ g OA in alum (results not shown).

In immunized animals, a blood pressure drop was the first response seen after challenge; it could sometimes be registered even in the absence of a BAR. Thus, it seems that the pressure drop may be used as an indication of a proper sensitization. However, this observation has not been pursued further.

Pauwels et al (1979c) found that the optimum antigen dose for the induction of an IgE antibody response differed from strain to strain. The highest degree of primary OA-IgE responses in BN, Wistar and PVG rats when Bp was employed as an adjuvant was observed at an immunization dose of 1 μ g OA, while Lewis rats showed a maximum in the primary OA-IgE antibody response at 100 μ g OA. In HL rats, Jarrett & Stewart (1974) showed that the lowest OA immunization dose, which together with Bp induced the production of reagin antibodies, was 1 μ g and that above that limit, the primary response was unaffected by the amount of antigen given. We therefore characterized the dose-response relationship for sensitization and provocation with OA, (with special reference to the temporal development of the capacity to mount a BAR) employing alum, Bp or silica as adjuvant.

As shown in Paper I, Fig. 2, we found that the BAR as well as the provocation dose of OA in SD rats differed with the immunization. The optimum provocation dose in animals immunized with 1 or 10 μ g OA 14 days before test was 0.5 mg OA, in animals immunized with higher doses it was 5 mg OA. Animals immunized with 100 μ g or 1000 μ g OA responded less well to low provocation doses of antigen. In 0.1 μ g OA- immunized animals, only a few individuals would respond to OA challenge. We therefore studied animals immunized with 1, 10 or 100 μ g OA with respect to temporal development of response capacity. The animals were

first challenged with a suboptimal dose (0.3 mg OA) followed by a maximum dose (5 mg OA) of antigen. It was not possible to give more than two provocation doses because of the great individual variation in sensitivity. As mentioned above the response to a 5 mg dose of OA is not affected by the previous response to the low antigen challenge dose. We noticed that the temporal development of the capacity to mount a BAR varied with the antigen dose used at immunization (FIGURE 4). Rats given 1 μ g OA responded particularly well to the low antigen dose at 3 weeks. This was followed by a stable response capacity until 6-8 weeks; after that the capacity to mount a BAR started to decline. Rats sensitized with 100 μ g OA developed a less pronounced BAR at 2 and 3 weeks; the reactivity had almost faded away at 6 weeks. In animals immunized with the intermediate dose of OA (10 μ g), the development of the capacity to mount a BAR apparently followed a dual time course characterized by an early short-lived peak of increased reactivity followed by a second more persistent phase of somewhat lower reactivity (FIGURE 4A,C). However, animals sensitized with 10 μ g or 100 μ g OA responded vigorously to the high challenge dose even at 7 weeks after immunization (FIGURE 4D). Animals immunized with 1 μ g OA together with 10 mg silica did not display a BAR at challenge, only those immunized with 10 μ g OA responded to antigen provocation (III, Fig. 4 or FIGURE 4). These animals showed a maximum response capacity at the low challenge dose 2 weeks after immunization; the reactivity had nearly disappeared at 7 weeks (FIGURE 4C,D). Finally, animals immunized with 1 μ g OA together with Perthydral showed a peak response at 5 weeks where the BAR was similar in magnitude to that of animals immunized with alum. However, at 7 weeks the reactivity to the low challenge dose of antigen in the former group of animals was somewhat reduced (FIGURE 4 A,B). The characteristics of the BAR in these animals were qualitatively similar to those of animals immunized with alum at LD.

Thus we found alum to be a suitable adjuvant for induction of a persistent BAR capacity. Moreover, by altering the antigen dose and the adjuvant employed at sensitization, different time courses for the development of the BAR capacity were recorded at a fixed challenge dose of OA. One explanation of these differences in response pattern with antigen dose and adjuvant em-

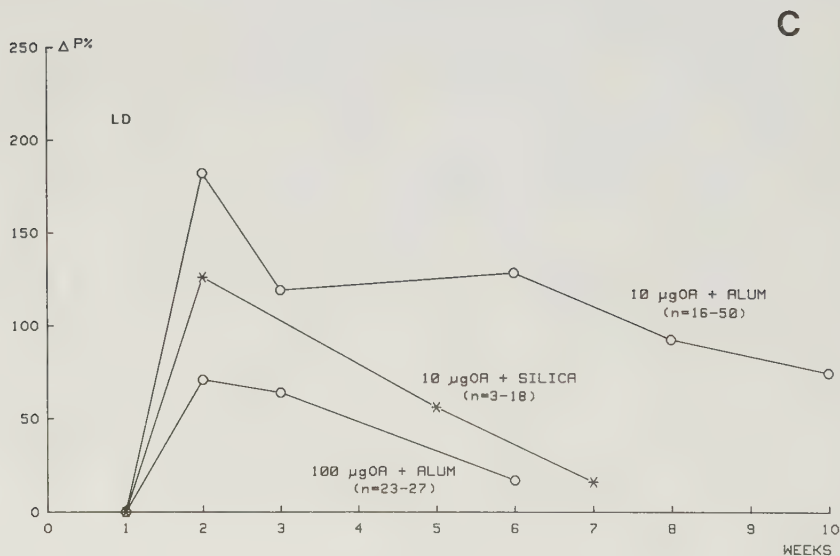
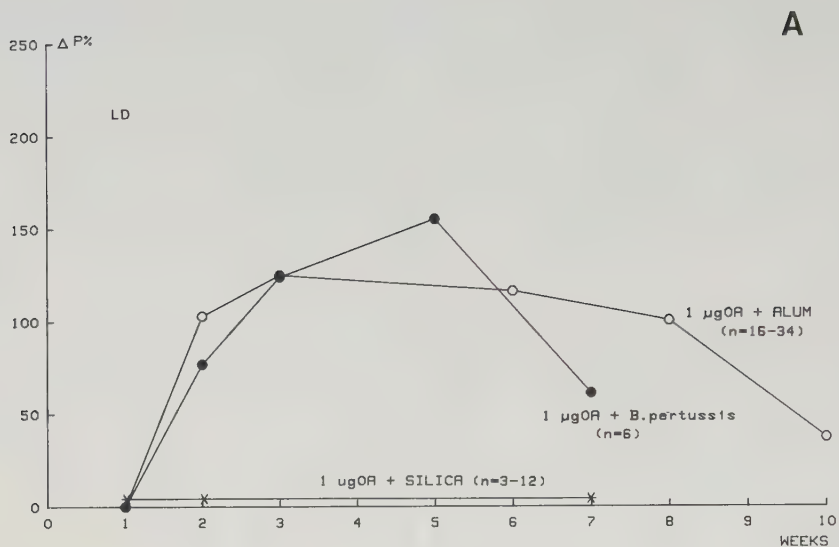


FIGURE 4 A, C

Temporal development of capacity to develop a BAR at different immunization regimens

Figure shows the temporal development of the capacity to react with BAR of animals immunized with 1, 10 or 100 μ g OA together with 100 mg $\text{Al}(\text{OH})_3$, 1 or 10 μ g OA together with silica or 1 μ g OA together with Perthydral. Challenge is performed with either LD; 0.3 mg OA (Frames A, C) or HD; 5 mg OA (Frames B, D). Figures in brackets denote number of observations.

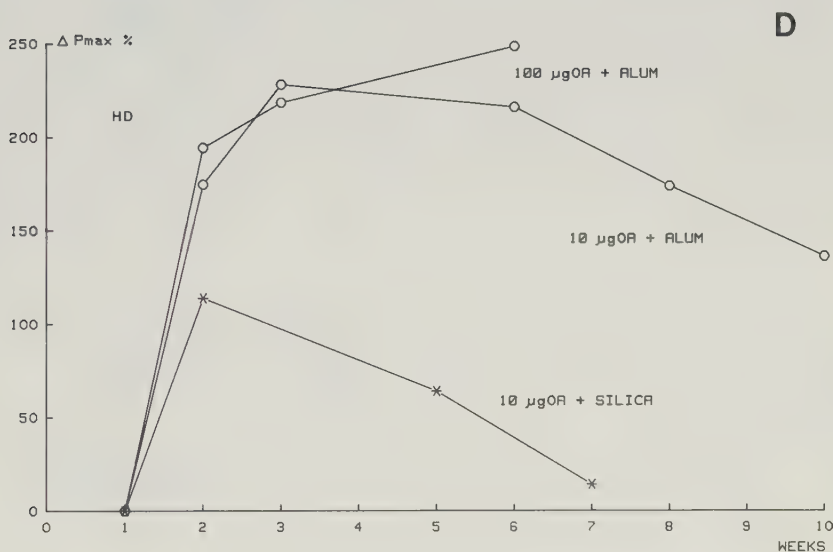
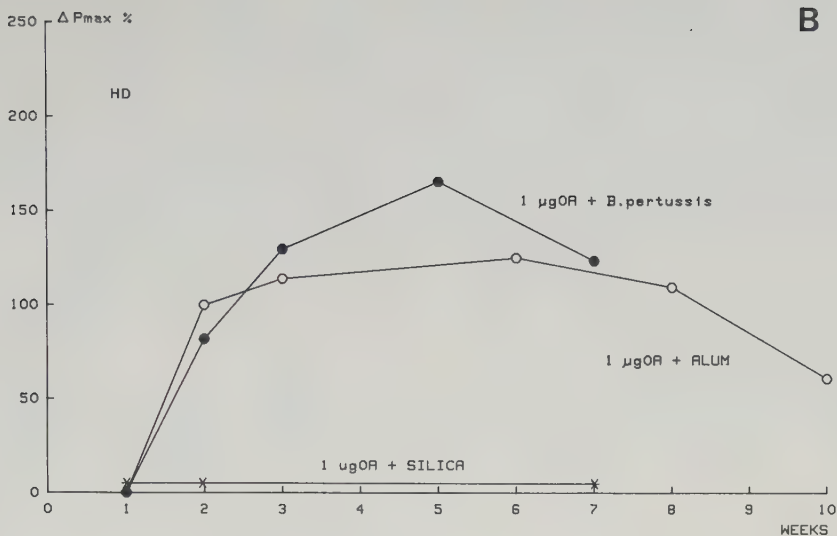


FIGURE 4 B, D

ployed at sensitization could be that more than one type of homocytotopic antibody mediates the BAR (see discussion in Section D).

The reproducibility in inducing a defined degree of BAR was also examined. We found that the response to a challenge with a

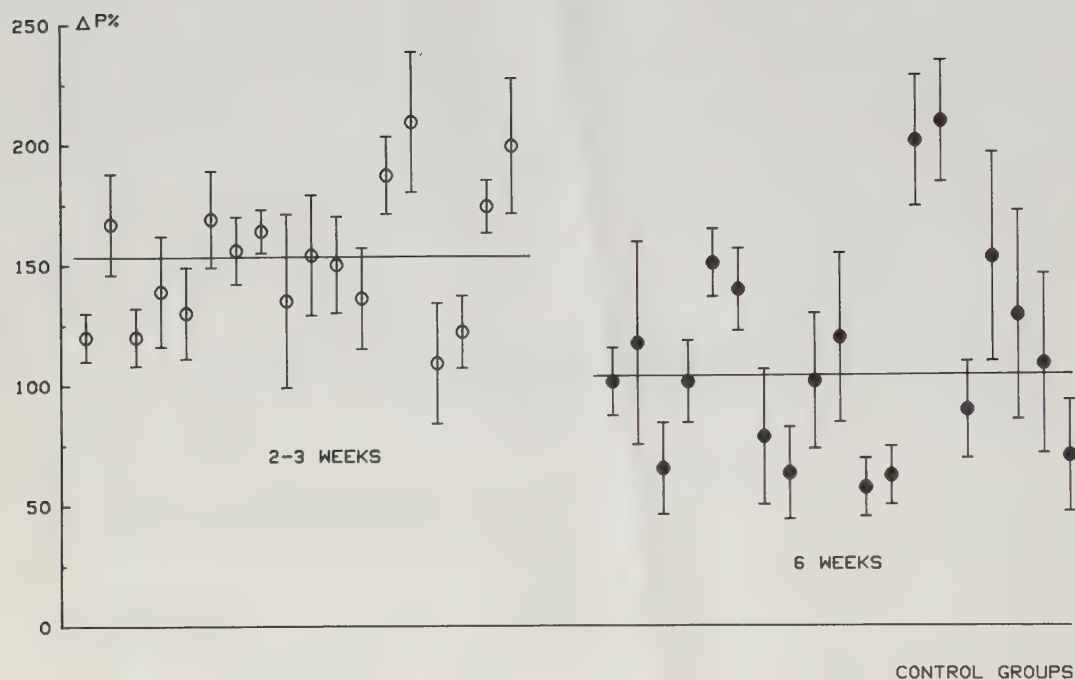


FIGURE 5

BAR of animals from different immunization groups
challenged with a low dose of antigen 2-3 or 6 weeks
after immunization

Mean BAR and SEM of animals from different batches immunized with 10 μ g OA and 100 mg alum and challenged with a low dose (0.3 mg) of OA at 2-3 weeks (open circles) and 6 weeks (closed circles) after immunization. Each point represents the results from 4-7 preimmunized animals.

defined dose of the antigen may differ between animals from different immunization batches. FIGURE 5 shows the BAR in animals immunized with 10 μ g OA and alum on different occasions. Challenge was performed two to three or six weeks after sensitization. The mean BAR varies significantly between immunization groups. Therefore, each experiment aiming at evaluating the effect of a drug has to have a control group of similar size being treated with vehicle. FIGURE 5 also shows that animals

tested at 2-3 weeks ($\Delta P\% \bar{x} = 153 \pm 13$) have a significantly ($P < 0.05$) higher mean BAR than animals challenged at 6 weeks after sensitization ($\Delta P\% \bar{x} = 108 \pm 16$) confirming the results in Paper I Fig. 3. The reason for the difference in BAR between immunization groups is not clear. However, genetic influence on the immunological response (the SD strain used is outbred), subclinical infections and the quality of the immunization as well as the quality of the adjuvant preparation used may vary from time to time.

C. Effects on the capacity to elicit a BAR by a secondary injection of antigen and/or adjuvant(s) with or without pretreatment of CY.

The primary or secondary IgE antibody response can be specifically modulated in various ways. Thus, Jarrett & Stewart (1974) found that when using different doses of antigen (1 μg or 1 mg OA) together with Bp in the primary immunization of HL rats, a booster injection of 1 μg OA without adjuvant could increase the PCA titre especially in animals primed with low antigen doses. BN rats immunized with a single dose of 10 μg OA together with 1 mg alum displayed increased specific IgE antibody serum levels when given a single booster injection of 10 μg OA without adjuvant 28 days later, but not when given lower doses, (Pauwels et al 1978). BN rats immunized with 10 μg or 100 μg OA together with 1 mg $\text{Al}(\text{OH})_3$ and boosted by repeating those doses with or without alum displayed enhanced levels of specific IgE antibodies (Murphey et al 1974, Pauwels et al 1978, 1979a). The last-mentioned results deviate from those of Jarrett et al (1980) in the respect that a booster response could be evoked although the adjuvant used in the priming event was alum.

We therefore examined the effect of booster injections of antigen on BAR capacity. Firstly, we found that low, repeated doses of antigen (0.5 or 5 μg OA) given together with alum or Bp did not increase the BAR capacity in SD rats (I). Secondly, animals given repeated injections of 1 μg OA alone 5 days a week for two weeks exhibited at best a low degree of BAR when challenged 10 days later. Thirdly, 3 to 5 injections of 1 μg OA together with 10 mg silica resulted in a weak response at the

low challenge dose. However, the response capacity in such animals was more pronounced and persisted longer than after a primary injection (I). Fourthly, and most interestingly, animals primed with 1 or 10 μ g OA together with 10 mg silica responded to a booster injection of a single dose of plain alum adjuvant given five weeks later with a markedly increased response capacity. A similar injection of plain Perthydral could not increase the BAR capacity that much (IV).

Taniguchi & Tada (1971) and Tada et al (1971) showed that rats pretreated with immunosuppressive drugs like cyclophosphamide (CY) or exposed to X-irradiation a few days before immunization developed increased and prolonged homocytotropic antibody responses. Similar experiences have been recorded by others both in rats and mice (Bennich et al 1978, Katz 1978). The enhanced IgE antibody production after this kind of treatment is thought to be due to the inhibitory effect of CY on antigen-specific and/or non-specific suppressor T cells (Chiorazzi et al 1976, 1977, Watanabe et al 1976). Andersson (1981) showed that guinea-pigs given CY either before primary sensitization or before a booster injection displayed an increased BAR when challenged with the antigen 9 weeks later. In another experimental setup in vitro, Bergstrand et al (1982) recorded an enhanced and prolonged histamine release capacity of lung tissue and serosal mast cells in rats given CY before the primary sensitization or before a booster injection.

We decided to examine the influence of CY treatment on the development of the BAR capacity. As SD rats are low IgE responders, CY treatment may be anticipated to markedly enhance their IgE response. In our experiments CY (35 mg/kg) given to SD rats two days before a primary injection of 1 or 10 μ g OA and alum did not markedly influence the BAR. Thus, CY could not prolong the high degree of bronchial response capacity seen at two weeks in 10 μ g OA immunized animals (I). On the other hand, in another experiment, the response to the high challenge dose 4 weeks after CY treatment tended to be increased (Paper I, fig. 4). Thus, our results indicate that under the chosen experimental conditions, treatment with CY does not influence the development of a BAR in SD rats.

D. Correlation between degree of BAR and serum antibody levels of IgE and IgG_{2a}.

It is well known that immediate hypersensitivity reactions are mediated by homocytotropic antibodies. In the rat these antibodies belong to the IgE or IgG_{2a} isotype. However, we are unaware of any demonstration in the literature of a clearcut relationship between serum levels of IgE or IgG_{2a} antibody to a specified antigen and the degree of immediate hypersensitivity reaction (allergic tissue reactivity) in this species. Many studies have been performed both in vitro and in vivo trying to show such a correlation (see e.g. Tables 1-3). Antigen-induced histamine release in vitro from mast cells, lung tissue, mesenteric tissue or skin does not uniformly correlate with serum IgE antibody levels measured either by PCA or by radioimmunoassay technique (Wilson & Bloch 1968, Pertillo & Smith 1973, Yamamoto et al 1974, Sydbom & Karlsson 1979). Studies in vivo also show a lack of correlation between antigen-induced bronchial anaphylactic reactivity and serum homocytotropic antibody titres (Stotland & Share 1974a, Church 1975b, Carswell & Oliver 1978b, Piechuta et al 1979, Ahlstedt et al 1983, Ufkes et al 1983). For example, in Wistar rats reinjected with Nb the serum IgE antibody level (PCA test) rose 8-fold. Despite this, the BAR was reduced by 50% (Church 1975b).

We therefore examined the relationship between the degree of BAR and IgE and IgG_{2a}-serum antibody levels to OA under the various conditions of immunization described above (Paper II). We were able to show that the degree of BAR in vivo was, in fact, correlated to the OA-IgE antibody levels under a few of the chosen conditions, namely (i) in animals immunized with 10 or 100 µg OA challenged with a high dose of OA 6 weeks after immunization and (ii) in animals given 10 µg OA 2 weeks before challenge with a low dose of OA. Under no other conditions did we observe a significant correlation, despite the presence of high serum OA-IgE antibody levels and a considerable degree of BAR (II). One explanation of the lack of correlation could be that late after immunization most IgE antibodies are connected to the mast cell receptors and therefore low levels of serum IgE antibodies do not reflect the degree of tissue sensitization.

Thus, Sydbom & Karlsson (1979) suggested that a longer half life of tissue-bound IgE than serum IgE might mask a correlation. Such a situation would explain our data in 10 μ g OA immunized animals where the OA-IgE serum antibody levels decreased with time whereas BAR following a low challenge dose was high at two weeks and then persisted at a slightly lower level. Note, however, that the opposite response was shown after the high challenge dose (II, fig 2). Another explanation of the lack of correlation may be that non-specific IgE competes with specific IgE for the IgE receptors (Zeiss et al 1978, Jarrett et al 1980). However, the following observations do not conform to the above mentioned explanations. Animals immunized with different doses of OA (1, 10 or 100 μ g OA) and examined at six weeks showed increased OA-IgE antibody levels and increased log ratios of OA-IgE/total IgE with increased sensitization dose, whereas the BAR following a low challenge dose of OA decreased with increasing immunization doses (II, Fig. 3). Such a drop in BAR with increasing sensitization doses is analogous with the reduction of serum IgE antibody levels seen, with increasing immunization doses, by Okumura & Tada (1971) and Ishizaka & Ishizaka (1978). These authors suggested that large doses of antigen used for primary sensitization activate antigen-specific suppressor T cells, which are responsible for the early termination of the primary response.

An unexpected finding was made in the group of rats which were primed with 1 μ g OA in 10 mg silica and given a booster injection of plain alum adjuvant five weeks after sensitization (III). A significant increase in the OA-IgE antibody level was seen in the alum-treated animals two weeks after booster. However, little change was recorded in the level of total serum IgE. At that time the BAR to a low challenge dose was increased; it correlated to the OA-IgE antibody level. In rats primed with a higher dose of OA (10 μ g) together with silica and given a booster dose of plain alum at 5 weeks, the OA-IgE antibody levels also increased and continued to rise until two weeks after booster. However, in these animals no correlation at all was seen between OA-IgE antibody level and BAR despite a high BAR (III, Fig. 3a, table I).

In the same study, another group of animals, boosted with plain Perthydral, showed increased total serum IgE levels two weeks later without any change in the specific serum OA-IgE (III, Fig. 3c). Only minor effects were seen on the BAR capacity. Similar findings were recorded in an in vitro study on antigen-induced histamine release from serosal mast cells or lung tissue in PVG rats by Bergstrand et al (1983). Pauwels et al (1983b) showed that BN rats injected with Bp, with or without a small dose of alum, showed enhanced IgE antibody production to an unrelated antigen, even if the primary antigen was given 6 weeks beforehand. These results also provide the important indication that alum and Perthydral interact in different ways with an (ongoing) IgE (antibody) synthesis.

Homocytotropic properties in the rat are connected not only to IgE but also to the short-term sensitizing antibody of the IgG_{2a} isotype (Bazin & Pauwels 1982). Could this antibody partly mediate the BAR 2 to 3 weeks or 6 weeks after immunization? To examine this we immunized SD rats with OA together with FCA (an adjuvant which activates the production of an IgE-suppressive factor and which is thought to favour IgG antibody production). Such animals did show a BAR at challenge with a low dose of antigen. However, we found that the BAR at the low challenge dose did not correlate with the OA-IgG_{2a} antibody levels. On the other hand, at the high challenge dose IgG_{2a} might clearly contribute to the mediation of the BAR (II, Table 2). Smedegård et al (1984) also failed to find a correlation between serum levels of IgG_{2a} antibodies and the degree of BAR.

These studies thus indicate that a highly significant correlation between BAR capacity and specific OA-IgE antibody levels can be recorded under certain but not under other conditions of immunization. Possible reasons for the lack of uniform correlation between BAR and specific OA-IgE or OA-IgG_{2a} serum levels have been discussed in Paper II and by Bergstrand et al. (1985b). These reasons include the possible existence of a functional heterogeneity in the rat IgE antibody isotype. The observation supporting this hypothesis includes differences in the protecting capacity of TERB, DSCG, THEO and D4026 with immunization conditions (I,IV) and in biphasic temporal pattern

in the log ratio of specific IgE versus total IgE in 1 and 10 μ g OA immunized animals (II).

E. Effects on BAR of anti-allergic and/or anti-inflammatory drugs

We were interested in characterizing the rat BAR model by examining the inhibitory capacity of four main types of anti-asthmatic drugs, i.e. a β_2 -receptor stimulating agent, representatives of xanthine derivatives, DSCG and glucocorticoids. As a β_2 -agonist we used TERB, as xanthine derivatives, THEO and the potent antianaphylactic experimental drug 3,7-dihydro-1,8-dimethyl-3-phenyl-1H-purine-2,6-dione (D4026) and as glucocorticoids BUD, DEX, and hydrocortisone.

a. Terbutaline

At the examined dose level (0.05 mg/kg), TERB, when given i.v. 1 min before challenge to rats immunized with 1 or 10 μ g OA and alum 6 weeks before challenge, was found to reduce the BAR in animals immunized with 10 μ g OA, only when challenged with a low and not with a high antigen dose (I). However, when given i.v., the drug was not effective in similarly immunized animals challenged 2-3 weeks after immunization (I). When given locally, either i.t. or by aerosol, TERB reduced the BAR induced by a low challenge dose of allergen almost as well when given at 2-3 as at 6 weeks after immunization. Again, no protection against the BAR induced by a high challenge dose of OA could be recorded with TERB under these conditions, except with i.t. administration (VI).

b. Xanthine derivatives

There is little information in the literature on the anti-anaphylactic effects of xanthine derivatives in the rat. However, Piechuta et al (1979) examined the protecting effect of THEO (100 mg/kg p.o.) on dyspnea symptoms and found this dose effective. We found that THEO, administered i.v. at the dose level 0.3 mg/kg, which is just below the required dose for bronchodilatation in the rat, was ineffective in reducing the BAR

induced by a low challenge dose of antigen. If anything the BAR was increased in animals treated with THEO (I). This contrasts with the situation in guinea-pigs where non-bronchodilating doses of THEO express marked anti-anaphylactic capacity (Andersson 1980b). At the dose level 1 mg/kg, THEO given i.v. produced a significant protection against a BAR induced by a low antigen dose; as for TERB the effect tended to be increased in animals examined late after immunization compared with the effect in animals examined early (VI). Xanthine derivatives are of great value in the treatment of asthma when given either p.o. or i.v., but inhaled they are of minor benefit for the patient compared with beta-adrenergic bronchodilator aerosols (Horton 1966, Stewart & Block 1976, Bohadana et al 1980, Cushley & Holgate 1985). Therefore, we also studied the effect of THEO given i.t. or by aerosol (VI). We found that i.t. administered THEO partially reduced the BAR induced by a low antigen challenge dose but with a flat dose-response relationship. When given as an aerosol THEO again partially inhibited the BAR.

D4026, when given i.v., showed a better capacity to inhibit BAR in animals challenged 6 weeks after immunization than in animals examined earlier. D4026 was more potent than THEO and displayed a marked inhibitory capacity even against the high challenge dose (I). D4026 was at least 100 times more potent than THEO with i.t. administration; it was in fact the most effective of the drugs examined with this route of administration with respect to reducing the BAR induced by a high challenge dose of antigen. D4026 was also effective with aerosol administration (VI, Fig. 3). However, because of toxicological findings in animal experiments (testis atrophy occurred at doses $\geq$ 6 mg/kg Dahlbäck et al 1984), D4026 has been excluded from clinical evaluations.

c. Disodium cromoglycate

In previous work (see, e.g., Table 3a,b), DSCG given either i.v. or i.t. has been found by most workers to be effective in protecting against a BAR in the rat (Church et al 1972, Stotland & Share 1974b, Farmer et al 1975, Carswell & Oliver 1978a,

Piechuta et al 1979, Ufkes & Ottenhof 1984). Some authors found a bell-shaped dose-response relationship for DSCG while others reported a linear relationship. An explanation of the bell-shaped dose response curve may be that an IgE mediated response is inhibited by DSCG whereas DSCG potentiates an IgG-mediated response (Church and Gradidge 1978). We found that 1 mg/kg DSCG i.v. reduced the BAR more effectively in rats challenged with a low antigen dose at 6 weeks, than in rats challenged at 2-3 weeks after immunization. No inhibitory effect could be recorded on the high antigen challenge dose (I). We also checked the effect of the same dose by only giving an HD but found no change in protecting efficacy compared with when the preceding LD was given. Like TERB, DSCG when given i.t. reduced the BAR independently of the time after immunization. DSCG given as an aerosol did not affect the BAR (VI).

d. Glucocorticosteroids

Andersson & Brattsand (1982) demonstrated, provided that the drug was given one day before challenge, that BUD or hydrocortisone reduced the BAR which is mediated presumably by an IgE-antibody in guinea-pigs, but not that mediated by an IgG type of antibody. Similarly, in a study by Church (1972) in Nb sensitized Wistar rats, DEX displayed its maximum protective activity after a 24 h pretreatment period. Stotland & Share (1974b) observed that DEX (1 or 10 mg/kg given p.o. at 18 and/or 1 h before challenge) did not significantly influence the BAR. An explanation of the poor inhibitory effect seen by Stotland and Share (1974b) with DEX could be that the provocation dose (10 mg OA) used by these authors was too high. Thus, we noticed that GCS showed only a weak inhibitory effect after the high challenge dose (5 mg OA, V). However, a significant reduction was recorded by Share & Stotland (1974b) when DEX was given 3 days and 1 h before challenge.

In our rat model we observed that BUD and DEX given i.p. could inhibit the response at the low challenge dose but only marginally at the high one. These effects of the GCS on the BAR were seen independently of immunological conditions (V). Furthermore, there was no marked difference in activity after i.p.,

i.t. or aerosol administration of these drugs. This data is in agreement with results found in a Sephadex-induced rat model of lung oedema, where BUD shows similar inflammatory inhibition irrespective of whether it was given i.v., i.t. or by aerosol (Brattsand et al 1985b). However, BUD (2.5 mg/kg i.t.) gave its maximum protection at 18-24 h. Hydrocortisone (2.5 mg/kg i.t.) showed a significant inhibitory effect (30%) only 1-5 h after pretreatment. No inhibitory effects were seen when BUD was given i.p. at a low dose (0.1 mg/kg) 1 min before challenge or when given repeatedly for three weeks (V). Previous work in the rat by Church et al (1972) showed DEX given p.o. to be five times less effective than when given i.p. When BUD and DEX were given by aerosol and the rats were challenged 4-7 h later, we found that both drugs reduced the BAR to the same degree (40% reduction of BAR). However, when challenge was performed 18-24 h after treatment DEX showed an increased (73%) and BUD a decreased (17%) capacity to inhibit the BAR. The maintained protection with DEX after 24 h could be due to the longer plasma half-life of this drug (Duggan et al 1975, Pauwels & Van Der Straeten 1982), whereas the rapid lung absorption and the shorter half-life of BUD could explain the lack of effect seen one day after treatment with this drug (Brattsand et al 1981, Ryrfeldt et al 1982, VI).

We also examined a combination of BUD and DSCG and found they exerted an enhanced protective effect in animals immunized with 10 µg OA and challenged at 6 weeks. No such effect was present when animals were challenged at 2-3 weeks (V). This finding corroborates that reported by Stotland & Share (1974b) when they combined DEX and DSCG and established a synergistic effect in diminishing the BAR. The reason for this synergism is not clear. However, from previous work by Church (1975a) it is obvious that the mechanism of action of the GCS on rat BAR cannot be mediator-antagonism. On the other hand, studies at the cellular level support the idea that the GCS may inhibit antigen-induced mediator release (Schleimer et al 1981, Marquard & Wasserman 1983, Bergstrand et al 1984). Ottenhof et al (1985) showed that this idea has been strengthened by the findings that prednisolone inhibits antigen-induced mediator release from isolated rat lung. The mechanism behind the enhanced inhibition seen by

combining DSCG with a GCS could be either that both act (at least in part) by an "anti-allergic" effect mechanism or that GCS may reduce increased permeability induced by released mediators at the endothelial cell level (Björk et al 1985, Svensjö & Roempke 1985) and thereby markedly strengthen the consequences of a mediator release inhibition induced by DSCG.

All the tested drugs demonstrated anti-asthmatic activity when applied i.t. However, this does not prove that they acted "locally" within airways as similar dose levels were required when given i.v. or i.p. (I,V,VI).

F. Characterization of mediators involved in the BAR

The relative importance, in the allergen-induced immediate and late phase reactions in man, of the various mediators derived from mast cells/basophils or from other inflammatory cells such as neutrophils and eosinophils is not yet clarified. The classical mast cell/basophil mediator, histamine, was initially thought to be responsible for the immediate anaphylactic airway response in man. However, since various antihistaminic drugs show limited efficacy in blocking such reactions and do not provide substantial relief from asthma symptoms, the role of histamine as a major mediator in asthma is questioned. Today the interest is focused primarily on lipid derived mediators such as leukotrienes (Piper 1984, Morris 1985b) and platelet-activating factor (Lynch et al 1984, Page et al 1985).

The role of various mediators in the allergen-induced immediate airway response varies from species to species and may also vary with the localization of the response in the airways. Thus e.g. antigen-induced BAR in guinea-pigs is mainly due to histamine with respect to reactions in the larger airways whereas leukotrienes seem to be primarily responsible for the reaction in the peripheral part of the lungs (Drazen et al 1979, Andersson 1982). Brunet et al. (1983) showed that in the rat, leukotrienes contracted the peripheral airways but not the trachea in vitro. Kinins may also take part in the bronchial response in guinea-pigs (Collier & James 1967).

The main mediator involved in the IgE mediated immediate BAR in the rat has been shown to be 5-hydroxytryptamine (serotonin) (5-HT) (Church et al 1972, Stotland & Share 1974b, Church 1975a, Farmer et al 1975, Brunet et al 1983). This fact is based mainly on the fact that methysergide, a 5-HT antagonist, partially blocked the BAR. Aas (1983) proposed that 5-HT could act either directly or indirectly via presynaptic receptors on cholinergic terminals in rat bronchi. We confirmed that methysergide effectively blocks the antigen-induced BAR. There was no indication that the efficacy of the drug varied with immunization condition (IV). Moreover, the airway reactivity to exogenously administered i.v. 5-HT was slightly higher in immunized animals than in controls. This data contrasts with that of Ahlstedt et al (1983) who in sensitized animals recorded reduced responses to 5-HT. Church (1975a) and Brunet et al (1983), suggest that an increased nonspecific bronchial hyperreactivity is not induced in sensitized rats.

Although allergen challenge of rat mast cells of the connective tissue type induces the release of both histamine and 5-HT (Morrison 1975) only 5-HT seems to mediate a BAR. An isolated perfused rat lung releases 12 times more histamine than 5-HT after antigen-induced bronchoconstriction. SRS-A was also detected in the lung effluent (Ottenhof et al 1985). Histamine H_1 -antagonists like mepyramine are generally ineffective in reducing the rat BAR (Church et al 1972). Stotland & Share (1974b) reported that a high dose of mepyramine (30 mg/kg) could significantly reduce the BAR. However, the specificity of mepyramine for H_1 -receptors at this dose was not shown. Histamine seems to be without bronchoconstrictory effect even in rather high doses (Church 1975b, Farmer et al 1975, Ottenhof 1985). We found that 100 μ g/kg histamine was unable to induce a bronchoconstriction (IV). Thus, we confirm that histamine probably plays a very minor role in the rat BAR.

Atropine, a drug, which blocks the cholinergic muscarinic receptor, was reported to reduce effectively the BAR in SD rats (Stotland & Share 1974b). However, atropine (1 mg/kg) did not significantly modify BAR in Nb sensitized Wistar rats (Church et al. 1972). We confirmed that atropine (1 mg/kg) reduced the BAR

(IV). Again, no difference in the efficacy of the drug was observed with different immunization conditions or times for challenge (IV). We also noted a slight increase in the bronchial response to i.v. 50 $\mu\text{g/kg}$ ACh (but not to 100 $\mu\text{g/kg}$ ACh) in rats immunized with 10 μg OA and alum compared with that in non-immunized animals. Moreover, repeated doses of ACh, in immunized but not in normal animals, led to a slightly higher bronchial response (IV). Similar results were recently recorded in rabbits (Halonen and Kaliner 1984). This data suggests some kind of increased cholinergic bronchial reactivity in sensitized animals, the basis of which, however, is not clarified. On the other hand, Church (1975a) and Brunet et al (1983) did not observe an increased reactivity to ACh or methacholine in immunized compared with normal animals.

Exogenously administered bradykinin induced a bronchial constriction in a dose-dependent way in rats (Church 1975a). Results contradicting this were reported by Farmer et al (1975) who found no such effects. DEX (5 mg/kg) could reduce contractions induced by bradykinin (Church 1975a). We observed that the degree of bronchoconstriction of bradykinin (at 400 $\mu\text{g/kg}$) did not differ between normal and sensitized animals (IV).

AA is released from various membrane-localized phospholipids such as phosphatidylcholine, phosphatidylethanolamine or phosphatidylinositol, mainly by an appropriately activated phospholipase A_2 enzyme activity (Irvine 1982). AA will then be metabolized either along the cyclooxygenase pathway resulting in end products such as prostaglandins and thromboxanes, or along the various lipoxygenase pathway(s) with end products such as leukotrienes (LTB_4 , LTC_4 , LTD_4 , LTE_4) (for reviews see Bisgaard 1984, Morris 1985b). Some of these products are potent bronchoconstrictors e.g. LTC_4 , LTD_4 , LTE_4 , TxA_2 , $\text{PGF}_{2\alpha}$, others mainly bronchodilators e.g. PGE_2 and some act as chemotactic principles and secretagogues engaged in inflammatory reactions, e.g. LTB_4 . AA can also be administered directly to animals. However, it is not clarified as to whether or not the effects recorded with exogenous AA are due to its conversion along the pathways involved in the metabolism of endogenously formed AA (see e.g. Clancy et al 1983).

To try to unravel any involvement of AA metabolites in the rat BAR we have employed (i) agents known to interfere with the metabolism of endogenously generated AA and (ii) examined the effects of exogenously given AA.

We showed a dose dependent bronchoconstriction of exogenous AA when given i.v. to rats. There was no difference between non-immunized and immunized animals in the bronchial response to AA when challenge was performed by a single dose of AA. However, normal animals given repeated doses of AA showed increased bronchoconstriction for the second dose compared with that of the first. This was not seen in immunized animals (IV, Table 2). We have no clear explanation of this difference. One could be that products of AA are more effectively metabolized in sensitized animals, another that sensitized animals are more effectively desensitized to the action of exogenous AA than normals.

With respect to the AA-induced bronchial reactivity we examined the inhibitory capacity of BW755C, I-B, INDO, NDGA and phenidone. All these agents, except NDGA, were found to partly block the AA-induced bronchial response. In a study on AA-induced respiratory distress in conscious rats, Parellada & Carbonell (1985) showed that BW755C inhibits whereas INDO failed to influence the respiration rate. The reason for the discordant effects recorded with INDO by these workers and ourselves is not clear.

We then turned to an examination of the effects of putative cyclooxygenase and lipoxygenase inhibitors and putative inhibitors of TxA_2 production on the antigen-induced BAR. We were interested in seeing whether any inhibitory capacity was in any way influenced by the immunological conditions. In a study by Ahlstedt et al 1983, INDO was previously shown to exert inhibitory effects on transpulmonary pressure in aerosol-sensitized rats. Contradictorily, Stotland & Share (1974b) and Farmer et al (1975) did not find any protecting effects with INDO (1 mg/kg) or aspirin (5 mg/kg) on BAR in actively sensitized rats. In in vivo experiments in OA-challenged rats INDO enhanced the SRS-A release into the peritoneal fluid of rats (Burka & Flower 1979). In guinea-pigs Andersson (1982) noticed that INDO enhanced an OA-induced BAR but strongly blocked an AA-induced

bronchial response. Similar effects were also seen by Burka (1983, 1985a,b) on antigen-induced tracheal contractions but not in parenchymal preparations in vitro. In our work INDO and I-B were found to inhibit BAR independently of immunization conditions and time for challenge. All these agents, except NDGA, were also found to partially block the AA-induced bronchial response (IV, Fig. 7).

In apparent contrast, we found that other compounds affecting the AA metabolism, i.e., BW755C and phenidone, tended to show varying inhibitory capacity depending on immunization conditions. Phenidone was more effective in inhibiting BAR in 10 µg OA than in 100 µg OA immunized animals whereas BW755C was more effective in animals immunized by 100 µg OA than in those given 10 µg OA (IV, Fig. 4). The reason for this is not clear. As mentioned above, NDGA unexpectedly failed to show any inhibition of BAR (IV). Piechuta & Holme (1982) observed in their OA-induced dyspnea model in unanaesthetized rats that NDGA and BW755C alone or in combination with methysergide would cause a dose dependent inhibition. In guinea-pigs, BW755C inhibited an antigen-induced BAR (Andersson 1982). However, in in vitro experiments in the same species, Burka (1983, 1985a,b) observed, that NDGA and phenidone inhibited contraction in guinea-pig airways while BW755C enhanced contractile responses in trachea at challenge with AA or antigen. BW755C, phenidone and NDGA were ineffective in a passive lung anaphylaxis model when using a high (100 mg/kg) challenge dose of OA (Lewis et al. 1983).

We also observed that combinations of methysergide and INDO or I-B almost completely blocked the BAR at both the low and the high provocation dose, and that combinations of methysergide with phenidone or NDGA did show enhanced inhibitory effect at the HD compared with methysergide alone (IV, Fig. 5).

PAF-acether, a phospholipid product containing an alkyl chain in 1-position, is a putative mediator of asthma and inflammatory diseases. It is released by a variety of immune and non-immune stimuli from e.g. mast cells, alveolar macrophages, basophils, neutrophils and platelets in experiments performed both in animals and humans (see Lynch et al 1984, Page et al 1985).

We examined the effects of exogenously administered PAF-acether on the rat bronchial response. We observed a dose-dependent bronchoconstriction with PAF-acether. Repeated doses of PAF-acether did not induce any response. This is probably due to a desensitization; similar experience has been reported by others (Camussi et al. 1983). PAF-acether (50 µg/kg) led to increased responses in some groups of immunized animals compared with normals. (IV, Table 1.)

PAF-acether-induced bronchial responses were less markedly affected by I-B, BW755C, INDO, and phenidone than were AA-induced responses (IV, Fig. 7). However, the PAF-acether-induced response was reduced by NDGA which is not the case with the AA-induced response. Thus, the PAF-acether-induced bronchial response in rats seems to involve AA products. Similar observations have been reported for PAF-acether induced bronchoconstriction in guinea-pigs (Lewis et al 1984).

In 1982 Piechuta and Holme summarized their studies of rat anaphylaxis. They concluded that both 5-HT and leukotrienes are involved in antigen-induced dyspnea in inbred rats.

Our results indicate that 5-HT and possibly acetylcholine are primary mediators of antigen-induced BAR in SD rats. Products of AA, derived from the cyclooxygenase pathway and possibly also by thromboxane synthetase, may also be involved in the allergen-induced bronchoconstrictory response.

The role played by the AA products is probably complex since the efficacy of inhibitors like BW755C and phenidone depends on immunization conditions whereas that of I-B and INDO does not. The inefficacy of NDGA in doses shown to affect the PAF-acether-induced bronchial response suggests that leukotrienes play a minor role in the antigen-induced BAR.

G. Effects of long-term treatment with drugs

In allergic disorders, the serum levels of IgE antibodies to the pertinent allergen(s) are usually elevated. A pharmacological reduction of high IgE antibody levels may therefore be a poss-

ible approach for treatment of allergic disorders (Katz 1984). Histamine, a mediator of the immediate type of allergic reactions is stored both in mast cells and basophilic leukocytes. Histamine has also been suggested to regulate immediate hypersensitivity and immune responses (Bourne et al 1974, Beer et al 1984). Baker and colleagues (1981) studied the effects of different histamine agonists and antagonists on the production of murine reagin antibodies in mice. They showed that H_1 -agonists enhanced the primary response while H_2 -agonists inhibited it and the opposite was found with appropriate antagonists. Andersson & Bergstrand (1984) demonstrated that long-term treatment with dimaprit, an H_2 -agonist, or a low dose of cimetidine, an H_2 -antagonist, reduced BAR mediated by IgE-like antibodies but not that mediated by IgG-like antibodies in the guinea-pig. They suggested that the mechanism underlying this inhibition was not a direct effect of the drugs on the airways. Asymptomatic asthmatic patients given a low dose of cimetidine once a day for 4 weeks were found to be significantly less responsive to bronchial allergen challenge during treatment than before. However, no changes in specific IgE antibody, total IgE levels or histamine release capacity were observed in either group (Bergstrand et al 1985a).

We were also interested in seeing if long-term treatment with H_1 -antagonists, H_2 -agonists or H_2 -antagonists could reduce BAR and/or diminish serum levels of OA-IgE, total IgE or OA-IgG_{2a} antibodies in sensitized rats. Groups of immunized SD rats (10 or 100 μ g OA + alum or 10 μ g OA + silica and boosted with alum) were given i.p. injections once a day five days a week for three weeks with dimaprit (0.1 mg/kg), cimetidine (0.1 mg/kg), metiamide (H_2 -agonist 0.1 mg/kg) or ketotifen (H_1 -antagonist 0.1 mg/kg), starting the fourth week after immunization. Drugs were withdrawn three to seven days before challenge. We found no alteration in any of the measured parameters for any of the drugs studied (results not shown). We also studied, in similar ways, the effects of long-term treatment with 5-HT (10 μ g/kg), its antagonist methysergide (1, 10, 100 μ g/kg), atropine (100 μ g/kg), INDO (3000 μ g/kg), DSCG (1000, 10000 μ g/kg) and BUD (10, 100 μ g/kg). Again, no effects compared with those of vehicles were recorded for any of the examined parameters. Long-term

treatment with dimaprit may have slightly increased the bronchial anaphylactic response capacity. DSCG (1000 µg/kg) and methysergide (1 or 10 µg/kg), if anything, slightly reduced the BAR capacity at the low dose, but it was only methysergide that tended to reduce the OA-IgE and OA-IgG_{2a} antibody levels (results not shown). As mentioned, none of the effects reached statistical significance.

GENERAL SUMMARY

This thesis describes in some detail the characteristics of the antigen-induced immediate bronchial anaphylactic reaction (BAR) in the SD rat. Special attention has been paid to (i) achieve conditions at sensitization and provocation permitting an optimal degree of BAR, (ii) the correlation of the BAR to serum levels of homocytotropic antibodies, (iii) the various mediators involved in the response, and (iv) the effects of widely used anti-asthmatic drugs.

For optimal sensitization to ovalbumin, alum was found to be a suitable adjuvant (i.p. administration of the inoculum proved effective). The dose of ovalbumin injected is of considerable importance. Thus, animals immunized with 0.1 µg OA only respond infrequently and then with a low degree of BAR. Animals injected with 1 µg or 10 µg OA respond well to a challenge with a low dose of antigen, only those given 10 µg OA show increased responses when challenged with a higher antigen dose. Both these groups of animals display their response capacity for a considerable time period after immunization. Animals immunized with higher doses of OA displayed a smaller BAR at the low provocation dose and their response faded away more quickly with time.

A significant correlation between the degree of BAR and the serum levels of OA-IgE, total IgE or OA-IgG_{2a} was only found under a few but not all of the examined experimental conditions. Thus, the degree of the BAR and the serum OA-IgE antibody levels correlated in 10 or 100 µg OA-sensitized animals challenged at six weeks with a high dose of OA and in 10 µg OA-immunized animals challenged at two weeks with a low dose of OA. There was

no correlation between the degree of BAR and OA-IgG_{2a} antibody levels in animals challenged with the low dose of OA. The reason for this lack of correlation between BAR and total IgE or OA-IgE antibody levels under these experimental conditions is not clear. Several possibilities are discussed in this thesis, including the possible existence of a functional heterogeneity in the group of mast cell sensitizing antibodies.

Animals immunized with OA together with Perthydral as an adjuvant tended to develop a bronchial anaphylactic response capacity similar to that of rats sensitized with alum. Silica was considered a less effective adjuvant than alum and Perthydral.

However, a booster injection at five weeks of plain alum or Perthyral precipitates/enhances the bronchial anaphylactic response capacity and/or the production of antigen-specific or nonspecific IgE and/or IgG_{2a} at seven weeks. Thus, a dormant antigen-specific allergic response capacity can be elicited on exposure of the animals to a nonspecific adjuvant alone.

The primary mediator of the BAR in the rat was deduced to be 5-HT. ACh, products of the cyclooxygenase pathway of AA metabolism, and possibly products related to thromboxanes contribute to the response. The relative importance of the last mentioned mediators may vary with immunization conditions. Products of the lipoxygenase pathway probably play a minor role in the development of the response.

The BAR was reduced by either systemically (i.v.) or locally (i.t.) administered TERB, DSCG, or the xanthine derivatives THEO and D4026. When given systemically the inhibitory capacity of these drugs varied with immunization test conditions. This was not the case when they were given locally (i.t.). Following aerosol treatment, TERB and D4026 reduced BAR while THEO showed a partial inhibitory effect, DSCG was ineffective. GCS given the day before challenge reduced the BAR induced by a low provocation dose of OA but only marginally that induced by the high antigen dose. There was no difference in effect between i.p. or i.t. administered GCS on BAR. BUD and DEX delivered as aerosol reduced the BAR if given 4 to 7 hours before challenge but only

DEX, probably due to its longer half-life, showed an inhibitory effect 18 to 24 hours before provocation. Long-term treatment with histamine receptor agonists/antagonists or "anti-asthmatic" drugs did not affect BAR capacity or serum OA-IgE-, OA-IgG_{2a} antibody or total IgE levels when drugs were withdrawn.

We conclude that the antigen-induced immediate bronchoconstriction in suitably sensitized SD rats is a model which deserves further studies with regard to its capacity to predict the effectiveness of new drugs aimed at relieving asthma symptoms in humans.

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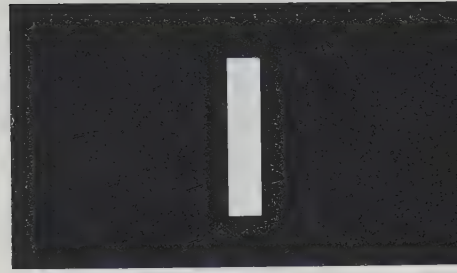
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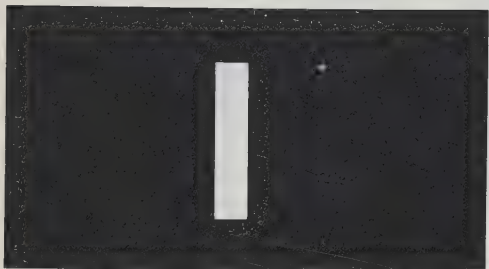
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Antigen-Induced Bronchial Anaphylaxis in Actively Sensitized SD Rats

Effects of Immunization and Provocation Doses of Antigen and of Pretreatment with DSCG, Theophylline, Terbutaline and a New Anti-Allergic Xanthine Derivative, D 4026

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Bronchial anaphylactic reactions, estimated as increase in intratracheal pressure, were precipitated by intravenous injections of antigen into actively sensitized SD rats. The degree of bronchial reactivity was found to depend on both the challenge dose and the immunization dose of antigen; therefore the course of the capacity to respond was recorded as a function of these variables.

The degree of the bronchial anaphylactic response could be reduced by pretreatment with disodium cromoglycate, terbutaline or a new anti-allergic xanthine derivative, D 4026, in some groups of animals. The efficacy of each agent was found to depend on the dose of antigen used for sensitization and for provocation of the bronchial reaction rather than on the strength of the response.

Taken together, the data suggest that more than one type of homocytotropic antibody mediates bronchial anaphylactic reactivity in the SD rat.

Key words: bronchial anaphylaxis; DSCG; ovalbumin sensitized rat; terbutaline; theophylline.

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CLINICAL ASPECTS

A rat model of asthma based on the principle of antigen-induced bronchial anaphylactic reactivity might more adequately be able to select agents with potential clinical usefulness in acute treatment. The present report describes the effect of varying immunization and provocation doses of antigen on the bronchial anaphylactic response capacity, measured as increased intratracheal pressure. Pretreatment of animals with DSCG, theophylline, terbutaline or D 4026, a new anti-allergic xanthine derivative, reduced the bronchial anaphylactic response in some of the immunized groups of animals.

Reagin-mediated allergic reactions are common asthma-precipitating events. The successful introduction in clinical practice of disodium cromoglycate (DSCG) stimulated interest in the development of new, orally effective "anti-allergic" agents for prophylactic use in asthma. This work primarily exploited the reagin-mediated immediate hypersensitivity reaction with

the rat skin and the release of histamine from peritoneal mast cells as discriminating tools. The disappointing clinical experience with several of the potent DSCG-like agents (for review, see 13) has clearly revealed the inadequacy of these models in their present design and has stressed the need of a reliable model of human extrinsic asthma in small laboratory ani-

* Subsidiary to AB Astra, Sweden.

Abbreviations used: DSCG, disodium cromoglycate; OA, ovalbumin; SD, Sprague-Dawley.

mals. The use of animal models of asthma based on the principle of antigen-induced bronchial anaphylactic reactivity would perhaps better select agents with potential clinical usefulness for acute treatment. Farmer et al. (18) claimed that inhibition of *passive* lung anaphylaxis in the rat is no more predictive as a screen for anti-allergic activity than passive cutaneous anaphylaxis. *Active* bronchial anaphylactic reactions have often been used as an instrument for drug selection. The guinea pig model has been discredited because IgG₁, and not IgE, is considered the major homocytotropic antibody in this species and DSCG is rarely effective in this model. However, Andersson (2) and Carney (7) have shown that bronchial anaphylactic reactivity in this species can be reduced by DSCG provided that immunization and challenge doses of antigen are carefully chosen.

Bronchial anaphylactic reactivity in rats, presumably due to the action of IgE antibodies fixed to lung tissue mast cells, has been studied by several authors. Church and coworkers sensitized animals by injection of the nematode *Nippostrongylus brasiliensis* (11, 12, 14). Church & Miller (15), like Stotland & Share (32, 33) and others (17, 26), used i.p. injection of ovalbumin (OA) in alum or *B. pertussis* (8, 9). Casey & Abboa-Offei challenged normal rats with anti-IgE (10), whereas passively sensitized animals were examined by Church (11) and by Farmer et al. (18). These studies indicate that the humoral factors involved in the mediation of human asthma and guinea pig and rat bronchoconstriction vary with the species used, but that the anaphylactic bronchoconstriction in the rat immunologically resembles that in human bronchial asthma (14).

Appropriately sensitized rats are known to be effective producers of IgE antibodies to various antigens, e.g. OA. However, it is well known that the antigen dose, the nature and dose of the adjuvant, the number of injections, the possible pretreatment with immunosuppressive drugs, the age of the rat, etc., have a profound influence on the degree and the kinetics of IgE antibody response (4, 21, 27–30). Furthermore, the antigen dose at challenge is an important

parameter determining the strength of the ensuing bronchial anaphylactic reaction and its inhibition by "anti-allergic" drugs both clinically and in bronchial anaphylaxis in guinea pigs *in vitro* (1, 2, 20). The purpose of the present study was to define, in SD rats, optimal conditions for induction of a persistent state of reagent-mediated anaphylactic reactivity and its provocation. We thought that such a model would be suitable in tests for acute and long-term effects of agents with potential anti-asthmatic effects.

MATERIAL AND METHODS

Animals and immunization

The animals used were outbred male Sprague-Dawley with a bodyweight of about 200 g obtained from Møllegaards Avlcentrum, Ejby, Skensved, Denmark. They were kept under conventional conditions. The animals were sensitized by intraperitoneal injections of ovalbumin (OA; Sigma grade III, crystallized and lyophilized) in 0.5 ml saline containing 100 mg dried and resuspended Al(OH)₃ (F 2200 Reheis Chemical Company, obtained through AB Astra, Södertälje), 10 mg amorphous Silica (Aerosil, particle diameter 25–50 nm, Degussa, Frankfurt am Main, obtained via Matak, Malmö, Sweden) or no addition of adjuvant. Selected groups of animals were given booster injections of OA alone or OA in Al(OH)₃, as specified in the text. In some groups of animals, the primary or the secondary injections of OA were preceded by i.p. injections of either 35 or 50 mg/kg cyclophosphamide (CY; Sendoxan®, Pharmacia, Sweden). Other groups of animals were given an injection of 10¹⁰ organisms of *B. pertussis* (SBL, Stockholm, Sweden) 4 days before test. Passive immunization was attempted by giving SD rats a volume of a specified serum intravenously (penis vein) at a varying interval before the test.

Respiratory measurements

At the indicated time after active or passive immunization, the animals were tested. Groups

of normal animals were also examined. The animals were anaesthetized with 50 mg/kg i.p. sodium pentobarbitone (Mebumal®, ACO, Sweden), succinylcholine chloride (Celocurin®, Vitrum, Sweden), 5 mg/kg, was administered i.p. to prevent spontaneous respiration. Respiratory measurements were made essentially as described by Stotland & Share (32).

The trachea was cannulated and the rat was ventilated with a Braun constant respiratory pump in a closed system. Tracheal pressure was recorded through a side-arm of the cannula connected to a Satham P23AC pressure transducer. The respiratory rate was set at 80 strokes/min with a tracheal pressure of 10 cm H₂O (tidal volume range 2.0–3.75 ml). The mean arterial blood pressure was recorded by a Satham P23AC pressure transducer throughout the experiment through a catheter inserted into the right carotid artery. The blood pressure pulses were transmitted to a tachogram, where the heart rate was registered. All recordings were made on a Grass polygraph model 7B. The animals were challenged with various doses of OA (5–5000 µg/animal) in 0.5 ml saline injected through a catheter inserted into the right jugular vein. The consequent increase in the tracheal pressure was used as an index of bronchoconstriction.

Selected groups of sensitized animals were treated i.v. 1 min before antigen challenge with one of the following agents: disodium cromoglycate (DSCG, a gift from Fisons, U.K.) in a dose of 1 mg/kg; theophylline (batch 11047, AB Draco, Sweden) in a dose of 0.3 mg/kg; terbutaline (AB Draco, Sweden) in a dose of 0.05 mg/kg, or 3,7-dihydro-1,8-dimethyl-3-phenyl-1H-purine-2,6-dione (D 4026, Draco, Sweden – a new anti-allergic xanthine derivative*) in a dose of 0.1 mg/kg. In these experiments, the animals were challenged with 300 µg OA followed a few minutes later by 5000 µg OA as described under Results. Separate experiments showed no difference in the strength or the characteristics of a bronchial anaphylactic response to the large challenge dose of OA whether or not it had been preceded by a chal-

lenge with a small dose of OA. Selected groups of animals, normal or sensitized, were also given serotonin (Sigma Chem. Co.) or metacholine (Sigma Chem. Co.) in a dose of 50 µg/kg, and 10 µg/kg, respectively, to induce bronchoconstriction.

All compounds, except D 4026, were dissolved and diluted in 0.9% NaCl (saline). D 4026 was dissolved in an equimolar dose of 0.5 M NaOH and diluted in 0.9% NaCl (saline).

Statistics

The figures recorded for bronchial anaphylactic response were found to be normally distributed, where a sufficient number of experiments were performed. Mean values and standard error of the mean were used for graphical illustration. Results of experiments for evaluating inhibitory capacity of drugs were statistically tested with analysis of variance.

RESULTS

Characteristics of response

At i.v. challenge with an adequate dose of OA, appropriately sensitized animals show an anaphylactic response in the form of an increase in intratracheal pressure (Fig. 1). The response is normally fully developed within 1–2 min. The arterial blood pressure is about 120 mmHg before challenge and decreases immediately after challenge to 50 mmHg. The heart rate is about 400 beats per minute before challenge, after challenge it decreases to subnormal values but soon recovers its initial ration.

Effect of varying immunization and challenge doses of OA on bronchial anaphylactic response 14 days after immunization

Groups of animals were injected with OA together with 100 mg Al(OH)₃. The dose given was either 0.1, 1, 10, 100 or 1000 µg OA. Fourteen days after immunization, the animals were used for testing. Each animal was chal-

* European patent application, publication No. 0001735.

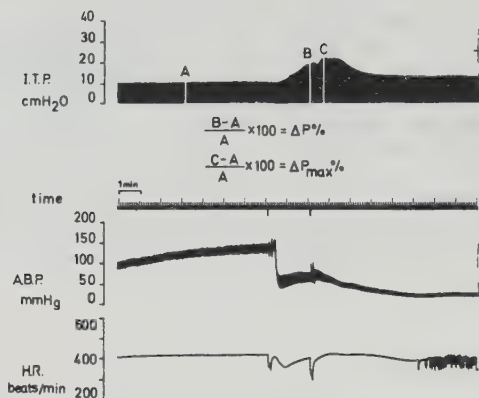


Fig. 1. Bronchial anaphylactic response to challenge with 300 μg OA (B) and 5000 μg OA (C) given i.v. to an SD rat immunized with 10 μg OA in 100 mg $\text{Al}(\text{OH})_3$ 14 days before test. The way of expressing the degree of the response ($\Delta P\%$) and the maximal response ($\Delta P_{\max}\%$) is indicated. Changes in arterial blood pressure (A.B.P.) and heart rate (H.R.) are also shown.

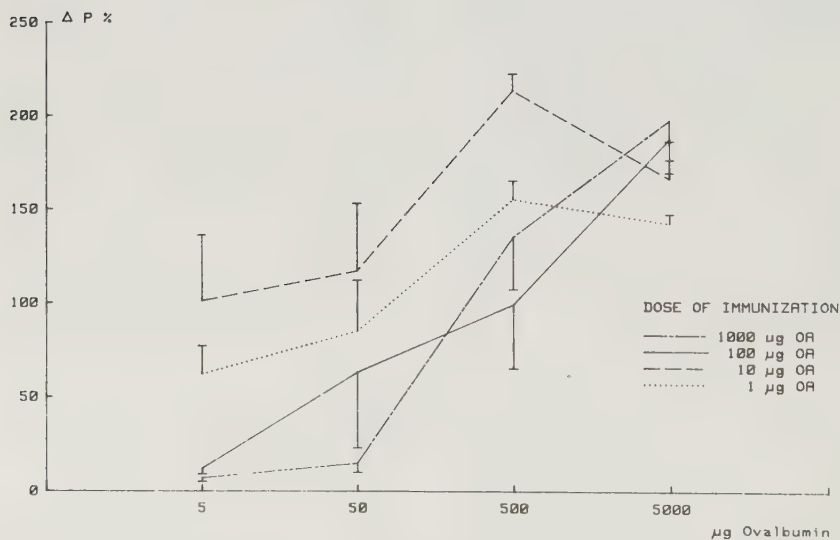


Fig. 2. Antigen-induced bronchial response ($\Delta P\%$) in animals immunized with 1, 10, 100 or 1000 μg of OA together with 100 mg $\text{Al}(\text{OH})_3$ 14 days before test. Challenge dose of OA is indicated on X-axis. Each point is the mean of five experiments. Verticals denote SEM.

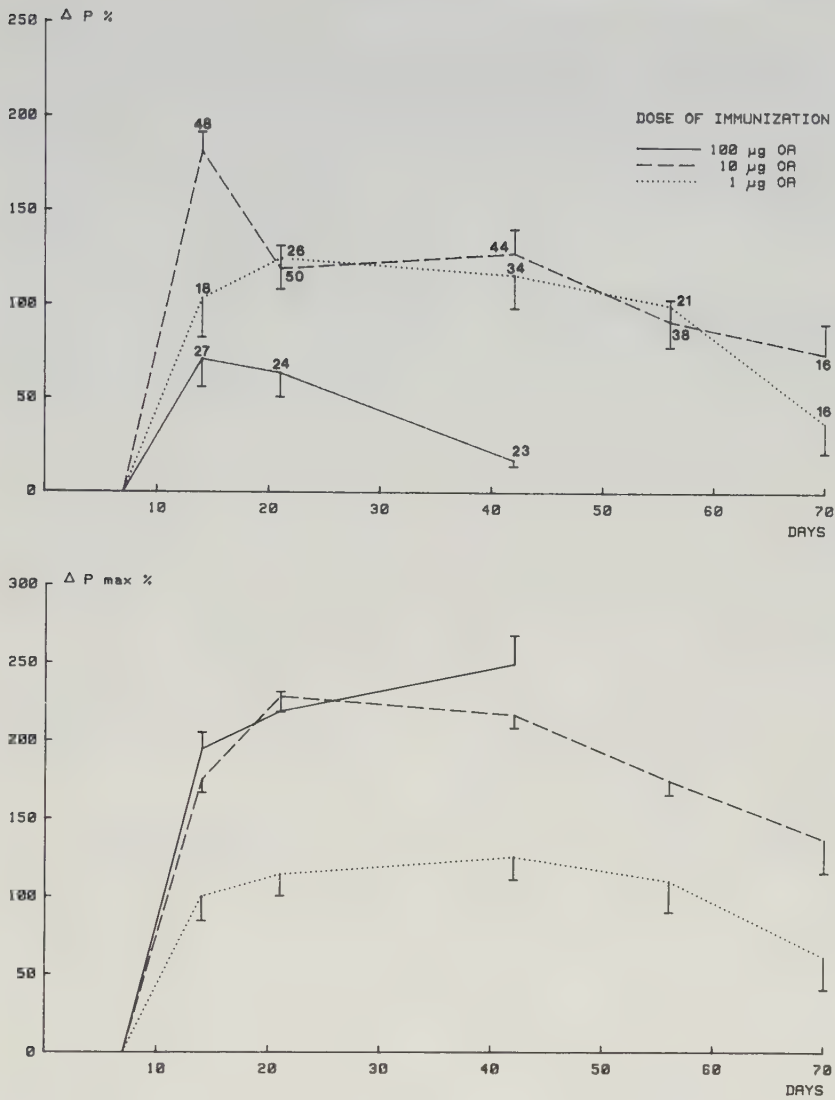


Fig. 3. Temporal pattern of development of bronchial anaphylactic response capacity in animals sensitized with 1, 10 or 100 μ g OA together with 100 mg $\text{Al}(\text{OH})_3$ and challenged with 300 μ g OA (frame A) or 5000 μ g (frame B). Figures denote number of observations at each point.

lenged with either 5, 50, 500 or 5000 μ g OA. A single dose of antigen was given to each animal. Fig. 2 shows the results recorded. Animals

given 0.1 μ g OA did not respond to any provocation dose. However, the rats sensitized with 1 μ g OA and especially those given 10 μ g OA

and alum reacted strongly on provocation with even comparatively small doses of OA (5 or 50 µg). Such animals responded maximally to 500 µg OA; dose of 5000 µg OA did not provoke any further increase in reactivity. Rats given 100 µg OA or 1000 µg OA in alum responded only weakly to small challenge doses but maximally to large ones.

Temporal development of capacity to mount a bronchial response in rats immunized with 1, 10 or 100 µg OA in alum or Silica gel

Animals immunized with 10 µg OA 20 days before the test were further examined with respect to the effect of the challenge dose. Only two out of nine animals given 50 and 150 µg OA responded with a pressure increase of more than 50%. All animals responded to 300 µg. Based on these observations, an initial challenge dose of 300 µg OA and a cumulative dose of 5000 µg OA were chosen to monitor the temporal development of the anaphylactic reactivity in rats sensitized with 1, 10 or 100 µg OA together with alum. The capacity to respond was examined at the indicated times from 7 to 70 days after immunization. Fig. 3a shows the results obtained with the small challenge dose. Rats given 1 µg OA developed a prolonged state of bronchial anaphylactic reactivity reaching peak level at 21 days and beginning to diminish 6–8 weeks after immunization. In animals sensitized with 100 µg OA in alum the anaphylactic reactivity was not as strong on day 14 and had disappeared by 6 weeks after immunization. The development of the response capacity in rats immunized with 10 µg OA in alum apparently reflects elements from two patterns; an early short-lived peak of increased reactivity followed by a longer phase of somewhat lower reactivity. Fig. 3b shows the responses to the large challenge dose. The course of the response capacity was similar to that in the animals given the small challenge dose of 1 µg OA. However, animals sensitized with 10 µg and particularly those given 100 µg OA responded more vigorously to the large challenge dose than to the smaller one.

Table 1 shows the correlation between the response to the small (ΔP) and that to the large challenge dose ($\Delta P_{\max}$). In the rats given 1 µg OA there was a highly significant correlation between these two parameters. However, in rats given 10 µg or 100 µg OA there was no such correlation except in the animals given 10 µg OA and tested on day 14 after immunization. We also examined the response capacity of animals pretreated with 50 mg/kg i.p. of CY 2 days before immunization with either 1 or 10 µg OA in alum. Such treatment did not clearly influence the bronchial response capacity. The effect of the use of Silica gel as an adjuvant was examined as well. Rats immunized with 1 µg OA and 10 mg Silica gel 28 days before test did not respond to the 300 µg OA provocation dose and only one out of three animals responded to 5000 µg OA. When immunized with 10 µg OA and 10 mg Silica gel 14 days before test, the animals responded to both the 300 µg OA and the 5000 µg OA challenge dose but the responses were not as strong as those observed in the animals immunized with the same amount of OA in alum (not shown).

Finally, eight SD rats were given 10 µg OA without any adjuvant. They were challenged after 14 days. None of the animals responded to the small (300 µg) or to the large (5000 µg) OA provocation dose.

Effects of secondary antigen injections with or without pretreatment with cyclophosphamide

Animals immunized with low doses of OA (0.1 or 10 µg) and alum were given repeated injections of 0.1 or 5 µg OA with or without alum and were challenged 4 or 14 days after their last injection. These animals did not differ from the controls.

The effect of treatment with CY on the secondary response in animals primarily given 10 µg OA and alum or 10 µg OA and Silica gel was then examined. The results are given in Fig. 4. In neither of these two experiments did CY-treated animals respond better to a secondary antigen injection than the animals given saline instead of CY. However, in experiment A animals given CY tended to respond better

Table 1

Correlation coefficients (r) recorded at calculation between response precipitated by a small (300 µg OA) and a large (5000 µg OA) challenge dose of antigen. The number of animals used is shown in brackets

	Time for test (day)					Immunization dose
	14	21	42	56	70	
Δ P % vs Δ P max %	0.81*** (18)	0.81*** (26)	0.90*** (34)	0.89*** (21)	0.81*** (19)	1 µg OA
Δ P % vs Δ P max %	0.76*** (48)	0.12 (50)	-0.08 (44)	0.29 (38)	0.35 (16)	10 µg OA
Δ P % vs Δ P max %	0.17 (27)	-0.10 (24)	-0.03 (23)	ND	ND	100 µg OA

*** P < 0.001.

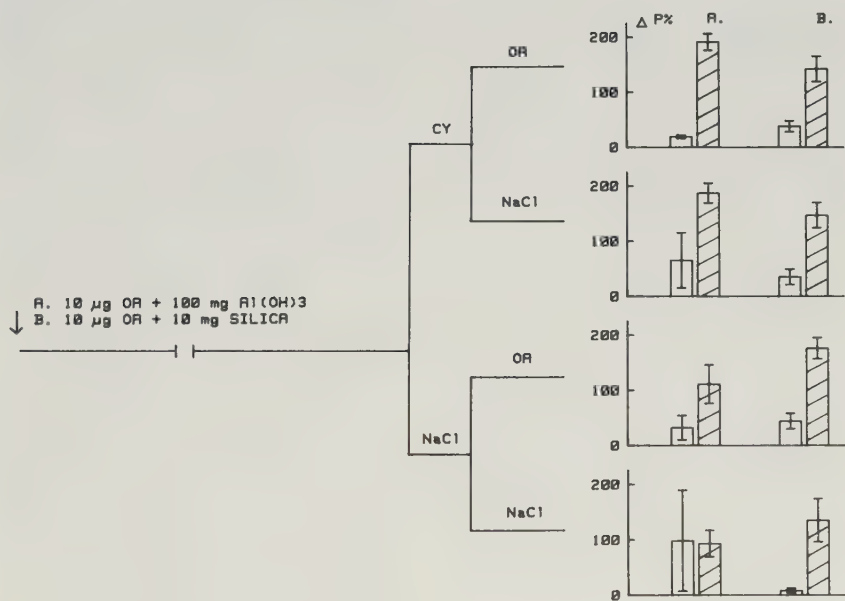


Fig. 4. Bronchial anaphylactic reactivity in rats primarily immunized on day 0 with 10 µg OA with Al(OH)₃ (experiment A) or Silica gel (experiment B) and boosted on day 61 (experiment A) or on day 30 (experiment B) with 10 µg OA with or without pretreatment 2 days before booster with 35 mg/kg i.p. of CY. Tests were performed 26 days after booster. Challenge doses of antigen: 300 µg OA (blank columns) and 5000 µg OA (hatched columns). Each value is the mean of four or five experiments. Columns denote mean of results from animals; verticals denote SEM.

to the 5000 µg OA challenge dose than animals given saline, irrespective of booster treatment.

Animals given 1 µg OA + 10 mg Silica on days 0, 28, 42, 56 and 70 were challenged 14 or 28 days after the third or the fifth antigen injection. No difference in the bronchial anaphylactic reactivity was found when the animals were given three or five injections of OA and Silica gel, but the responses persisted longer and the reactivity was higher than after a primary immunization.

A further group of SD rats were immunized by repeated injections of 1 µg OA without adjuvant 5 days a week for 2 weeks. They were challenged 10 days after the last injection. These animals responded weakly to the 300 µg OA challenge dose ($\Delta P\% = 30 \pm 13$, $n = 6$) and moderately to 5000 µg OA ($\Delta P_{\max}\% = 131 \pm 27$, $n = 6$).

Effect of treatment with B. pertussis on bronchial reactivity

An i.p. injection of *B. pertussis* was given to a group of rats 26 days after immunization with 1 µg OA in Al(OH)₃. No difference was found in bronchial anaphylactic response capacity between the animals treated with *B. pertussis* or saline 4 days after challenge (not shown).

Effects of sensitization on bronchial response to the constrictor serotonin and methacholine

Twenty-four SD rats were immunized, 12 with 1 µg OA in alum and the remaining 12 with 10 µg OA in alum. On days 25 and 56, six animals in each group and six normal animals were challenged with the bronchoconstrictors serotonin (50 µg/kg) and methacholine (10 µg/kg). In the doses used, both serotonin and methacholine induced suboptimal degree of bronchoconstriction in the normal animals. The immunized rats did not show increased bronchial reactivity compared with the controls to either of the two constrictors.

Attempted passive sensitization

SD rats with a bodyweight of 200 g were passively immunized intravenously, intraperitoneally or intramuscularly with 1 ml of a serum pool, obtained from rats actively sensitized with 10 µg OA in alum 19 days before harvest. Sera from such animals contain IgE-antibodies (3). The passively sensitized animals were challenged 72 hours after transfer, first with a small, and then with a large, antigen dose. None of them responded with changes in intratracheal pressure, but there was a clear fall in blood pressure from 120 mmHg to 50 mmHg.

Effect of anti-asthmatic agents on the bronchial anaphylactic response

Groups of animals immunized with 1 µg or 10 µg OA in Al(OH)₃ 21 or 42 days before being tested were treated with DSCG, terbutaline, theophylline or D 4026 or with saline as a control 1 min before challenge with a small (300 µg OA + 100 mg Al(OH)₃) the inhibitory effects of D 4026 and DSCG were less marked on day these doses had been found the most suitable. One to two minutes later, when the response to the small antigen dose was fully developed and had begun to decline (see Fig. 1), a new antigen challenge dose of 5000 µg OA was given. The effect of pretreatment with DSCG and D 4026 was also examined in animals immunized with 10 µg OA 14 days before the test. The experimental approach is exemplified in Fig. 5, which shows that in animals given 10 µg OA + 100 mg Al(OH)₃ the inhibitory effects of D 4026 and DSCG were less marked on day 14 than on day 42 despite similar bronchial anaphylactic reactions in the control animals on days 14 and 42. The effects of the drugs are summarized in Fig. 6. Theophylline, in a sub-bronchodilatory dose, did not effectively inhibit the bronchial anaphylactic reactivity in any of the test conditions. If anything, it tended to enhance the bronchial anaphylactic response in animals immunized with 1 µg OA 42 days before the test. DSCG, terbutaline and D 4026 all showed slight, inhibitory effects in animals examined 14 or 21 days after sensitization with

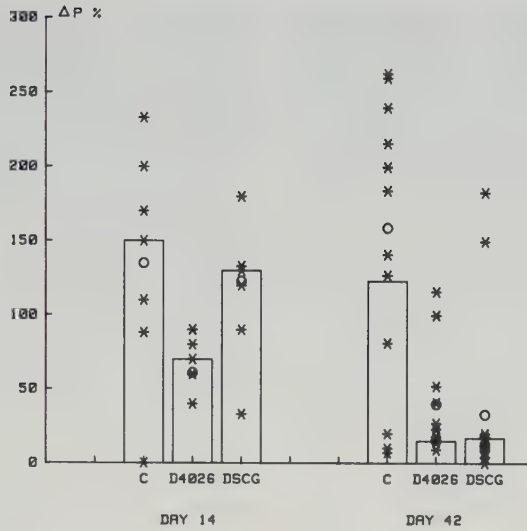


Fig. 5. Effects of D 4026, DSCG and saline (C) on the bronchial anaphylactic reactivity in animals immunized with 10 µg OA 14 or 42 days before test. The columns denote the median value; the circles, the mean value; and the asterisks the individual values.

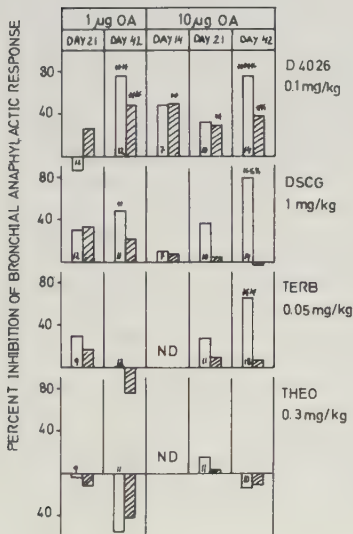


Fig. 6. Effects of DSCG, terbutaline, theophylline and D 4026 on bronchial anaphylactic reactivity in animals immunized with 1 µg OA 21 or 42 days or 10 µg OA 14, 21 or 42 days before test. Drugs were given at the indicated dose 1 min before the challenge dose of OA. Each column denotes mean percentage inhibition, i.e.

$$\left(1 - \frac{\text{mean } \Delta P \% \text{ in drug treated animals}}{\text{mean } \Delta P \% \text{ in saline treated controls}}\right) \times 100$$

Number of animals in each group is given in the Figure. Differences between groups were evaluated with analysis of variance. Blank columns denote results obtained when challenge was performed with 300 µg OA, shaded columns results obtained after a second challenge with 5000 µg OA to provoke a maximal response.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

either 1 or 10 μg OA, but the inhibition was statistically significant only for D 4026. DSCG, terbutaline and D 4026 significantly inhibited the bronchial anaphylactic response provoked by 300 μg OA – though only D 4026 was effective in a challenge dose of 5000 μg OA – in animals tested 42 days after immunization with 10 μg OA. In animals immunized with 1 μg OA, only D 4026 showed a significant inhibitory effect at this time. It should be observed that the degree of bronchial anaphylactic reactivity *per se* was not lower on day 42 than on day 21 after immunization (see Fig. 3). The experiments with terbutaline and with DSCG thus clearly indicated that the effect of an agent varies with the immunization dose of antigen and the time after immunization; e.g. terbutaline is a very effective inhibitor in animals given 10 μg OA but not in animals given 1 μg OA 42 days before the test.

DISCUSSION

The present report describes the development course of the capacity to react with a bronchial anaphylactic response at antigen challenge of rats immunized with graded doses of OA using IgE-antibody promoting immunization regimens. The course showed several unexpected characteristics. First, animals immunized with 10 μg OA tended to show a biphasic pattern of development of their capacity to respond to a small challenge dose of antigen. We suspect that this pattern is composed of two overlapping ones. The early part of the response in such animals, a high but rapidly declining reactivity, is probably similar to the type of response observed in the animals given 100 μg OA. These animals develop an early transient reactivity, as revealed by the small challenge dose. Second, in animals given 1 μg at immunization, but clearly not in animals given 10 or 100 μg OA, there was a significant correlation between the responses to the small and the large challenge doses (Table 1). This suggests three possibilities: 1) Either the response to the large challenge dose of antigen in the latter groups is mediated by a kind of antibody other than that which

mediated the small challenge dose response. 2) blocking antibody seriously influences the outcome of the challenge; or 3) systemic anaphylactic reactions influence the response. The first possibility is strengthened by the third main finding in the present investigation. One anti-allergic agent, DSCG, and one β_2 -agonist, terbutaline, in the concentration used are somewhat effective as inhibitors 6 weeks after immunization with 10 μg OA but not after 3. Besides its interesting theoretical aspects this observation should be of value to those who use the present model for testing anti-allergic DSCG-like drugs. Previous estimates of the inhibitory efficacy of DSCG in the present system vary from low to high (14, 18, 33); apparently different conditions at the test can explain the divergence in the results. In this connection it might not be out of place to mention that a bronchial anaphylactic reaction induced by anti-IgE antibodies is very susceptible to inhibition by DSCG (10).

There is one more comment to be made in connection with the inhibition experiments. According to our experience, when bronchoconstriction was induced by 50 $\mu\text{g}/\text{kg}$ serotonin, the threshold dose of theophylline for bronchodilatation was 0.5 mg/kg, while theophylline in a slightly smaller dose showed no anti-allergic properties at all. This contrasts with results obtained with a slightly different technique in guinea pigs (2).

The problem of identifying the antibodies mediating the response(s) will be dealt with in greater detail elsewhere (3). However, a few comments on experiments we performed to better define the rat bronchial anaphylactic response seem warranted.

First, Mancino & Bevilacqua (24) used Silica gel as an adjuvant in mice to induce a reaginic antibody response to OA apparently due only to IgE antibodies. This response could be boosted. We utilized Silica gel as an adjuvant and our results indicate that this agent effectively induces bronchial anaphylactic reactivity in SD rats after repeated injections. Similar results have been reported with respect to histamine release from lung tissue and mast cells (5).

Second, Taniguchi & Tada (34) made the important observation that in rats, treatment with immunosuppressive drugs or X-rays a few days before immunization with small doses of antigen delays but afterward enhances a homocytotropic antibody response. Similar results have been reported by others (for ref. see 4, 22). In the present experiments, CY given 2 days before a primary dose of 1 μ g or 10 μ g OA together with alum did not markedly influence the reactivity. In other words, it did not prolong the high response capacity seen on day 14 in 10 μ g OA-immunized animals. On the other hand, there was a slight increase in the response to a high antigen challenge dose 4 weeks after CY-treatment of animals immunized 8 weeks before the test (Fig. 4). This effect of CY is not influenced by a booster injection of antigen 2 days after the CY treatment. These results cannot be taken as evidence for or against IgE antibodies mediating this kind of bronchial anaphylactic reactivity. It is noteworthy that a slight enhancement of IgG antibody synthesis in rats has been reported by Gagner & MacLennan (19) using a slightly different schedule for CY administration.

Third, as shown elsewhere (3), when rats are sensitized to OA together with Freund's adjuvant, their response is only weak. Taken together with serum OA-IgG_{2a} antibody determinations these results clearly indicate that IgG_{2a} antibodies are of only minor importance as mediators of the response.

Fourth, our attempts to characterize the antibodies mediating the bronchial anaphylactic reactivity by heat-treatment of serum before passive transfer have been hampered by difficulties in transferring more than a slight degree of activity with fresh non-heated serum. In this respect our data seem to corroborate those of Church (11). He transferred response capacity with 4 ml reaginic sera (PCA-titre 1:60) i.p., but the degree of reactivity transferred was low. Farmer et al. (18) and Carswell & Oliver (8) reported transfer of reactivity with high titted reaginic sera, but they used a large challenge dose of OA (25 mg/kg) or 20 mg/ml aerosolized OA to provoke the reaction. Taken together, these data indicate that systemic passive transfer

of reagin-mediated bronchial anaphylactic reactivity in rats is much more difficult to achieve than transfer of systemic anaphylactic reactivity mediated by IgG antibodies in guinea pigs (6). However, passive transfer of reactivity is not impossible. Thus, SD recipients given 4 ml each i.v. of a fresh high titted antiserum against OA produced in BN rats (OA-IgE antibody titre 238 PU/ml) responded equally well as highly reactive, actively sensitized rats (Dahlbäck, unpublished). A clearer definition of the optimal conditions for passive transfer of reactivity in SD rats must await further research.

A major characteristic of bronchial asthma is nonspecific hyperreactivity of the airways to, for example, allergic mediators. The cause of this hypersensitivity is not known but environmental factors might be a contributory cause. Cockcroft et al. (16) have reported that exposure to allergens can raise the degree of hyperreactivity in some asthmatics. It is possible that an ongoing immune response affecting bronchial tissue might increase bronchial hyperreactivity. However, our results showed no increased bronchial reactivity to i.v. serotonin or methacholine in animals immunized with 1 μ g or 10 μ g OA 42 days before the test. Thus, a persistent anaphylactic response capacity does not seem *per se* to lead to increased bronchial nonspecific reactivity. In these respects our results corroborate those of Church (11) in the rat and those reported by Popa et al. (31) in guinea pigs.

In certain strains of rodents, *Bordetella pertussis* enhances anaphylaxis and/or the reaction to chemical mediators, e.g. serotonin and histamine (25). This has been considered an interesting model for studying nonspecific hyperreactivity in bronchial asthma. Our efforts to enhance an antigen-induced bronchial anaphylactic reaction by treatment of sensitized animals with *B. pertussis* 4 days before the test were unsuccessful. But it is not known why; it might simply be that SD is a strain that does not respond to *B. pertussis* or that the quality of the *B. pertussis* vaccine used was not good enough to induce hypersensitivity.

Summing up, the following observations of interest were made in the investigation of the

course of development of bronchial anaphylactic reactivity in SD rats sensitized according to IgE-antibody promoting regimens: (a) In one group of animals the response to a small challenge dose tended to be biphasic, (b) the correlations between the strength of the response to a small dose and a large dose of antigen were inconsistent and (c) the efficacy of the anti-allergic agents varied with the testing conditions. These observations suggest that more than one type of homocytotropic antibody might contribute to the response recorded.

ACKNOWLEDGEMENTS

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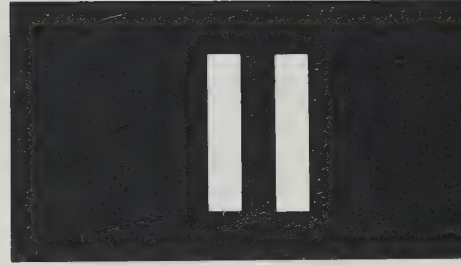
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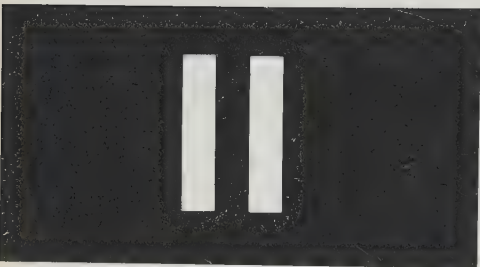
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Relation between Bronchial Reactivity to Antigen *in Vivo* and Serum IgE and IgG_{2a} Antibody Levels in Rats

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Sprague Dawley rats were immunized with graded doses of ovalbumin (OA) together with alum. The capacity of the animals to produce a bronchial anaphylactic response to intravenous antigen challenge was related to serum OA-IgE and OA-IgG_{2a} antibody levels estimated by radioimmunoassay. A significant correlation between bronchial anaphylactic response capacity and OA-IgE antibody levels was found under a few, but not all, of several carefully chosen experimental conditions. No correlation was demonstrable between the *in vivo* reactivity of the animals and their serum levels of OA-specific IgG_{2a} antibodies.

Key words: bronchial anaphylaxis; IgE and IgG_{2a} antibody levels; ovalbumin sensitized; SD rats.

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CLINICAL ASPECTS

It is now established in humans that immediate type allergic reactions are primarily mediated by IgE antibodies. The present investigation examines whether the rat is a suitable experimental model for studies of the correlation between bronchial anaphylactic response and IgE serum antibody levels. The results show that the degree of bronchial anaphylactic response was correlated to serum titre of OA-IgE antibodies only under certain well-defined circumstances but not to OA-IgG_{2a} antibodies.

It is now firmly established in humans that immediate type allergic reactions are primarily mediated by a special class of antibodies known as IgE. In allergic disorders, the levels of IgE antibodies to the pertinent allergen(s) are usually elevated. Pharmacological reduction of high IgE antibody levels should therefore be a possible approach to the treatment of atopic disorders (15).

The rat should conceivably be a suitable experimental animal in the search for agents with IgE antibody reducing properties, because when appropriately sensitized, rats produce IgE antibodies that can be accurately determined by radioimmunoassay. Furthermore, the animals are sufficiently large to permit relevant studies on the physiological significance of a reduced IgE antibody response.

However, the literature contains a number of reports indicating that there is no simple correlation in this species between the titre of IgE antibodies and the degree of allergic tissue reactivity. Thus, antigen-induced release of histamine *in vitro* from mast cells, lung tissue, mesenteric tissue, or skin does not necessarily vary with serum titres of IgE antibodies estimated by passive cutaneous anaphylactic (PCA) tests or radioimmunoassay (23, 27, 30, 31, 32). Similarly, the correlation between antigen-induced bronchial anaphylactic reactivity and serum reagin titres is not unequivocal (5, 6, 24, 29). Several possible explanations for these discrepancies have been suggested but the item is not clarified.

We recently described in some detail the temporal pattern of development of the bron-

chial anaphylactic response capacity *in vivo* in Sprague Dawley (SD) rats (9). The animals used in those studies were immunized according to various regimens chosen to stimulate preferentially IgE antibody synthesis. In the present investigation the degree of response in this test was studied for any correlation with the serum titre of OA-IgE antibodies, OA-IgG_{2a} antibodies, the level of total IgE, or the ratio OA-IgE antibody/total IgE.

MATERIAL AND METHODS

Animals and immunization

Outbred male Sprague Dawley (SD) rats of SPF status were used, preliminary experiments having shown this strain to be suitable for respiratory measurements. The animals were obtained from Møllegaards Avlcentrum, Ejby, Skensved, Denmark, weight about 200 g. They were reared under standard animal housing conditions. Sensitization was performed by either of the following two procedures.

A. Intraperitoneal injections of the indicated dose of ovalbumin (OA; Sigma grade III, crystallized and lyophilized) in 0.5 ml saline containing 100 mg of dried and resuspended Al(OH)₃ (F 2200 Reheis Chemical Company, obtained through AB Astra, Södertälje). At least 1 h before injection the adjuvant was resuspended in the antigen solution.

B. In each of the four footpads intracutaneous injections of 0.05 ml were given of an emulsion of equal parts of OA-solution and FCA (Difco Lab, Detroit, Michigan).

Tests of anaphylactic reactivity in vivo

Respiratory measurements

At the indicated time after immunization, the animals were taken to test. They were anaesthetized with 50 mg/kg i.p. pentobarbital (Mebumal®, ACO, Sweden); 5 mg/kg succinylcholine chloride (Celocurin®, Vitrum, Sweden) was administered i.p. to prevent spontaneous

respiration. Respiratory measurements were made essentially as described by Stotland & Share (29).

The trachea was cannulated and the animal was ventilated with a Braun constant respiratory pump in a closed system. Tracheal pressure was recorded through a side arm of the cannula connected to a Statham P23AC pressure transducer. The respiratory rate was set at 80 strokes/min with a tracheal pressure of 10 cm H₂O (stroke volume range 2–3.75 ml). Throughout the experiment the mean arterial blood pressure was recorded by a Statham P23AC pressure transducer via a catheter in the right carotid artery. The blood pressure pulses were transmitted to a tachogram, where the heart rate was registered. All the recordings were made on a Grass polygraph model 7B. The animals were challenged with the intravenous injection of a specified dose of OA in 0.5 ml saline through a catheter in the right jugular vein. 300 µg OA was injected initially. The resulting increase in the tracheal pressure was recorded. As soon as this response had developed (within 1–2 min) 5000 µg OA was injected and the resulting response was taken as the maximum attainable. Separate experiments showed that the preceding injection of 300 µg OA under the present circumstances did not influence the response to the 5000 µg dose. We are aware of the fact that this does not apply to bronchial anaphylactic reactions in the rat under all experimental conditions.

OA-IgE antibody and total serum IgE levels were measured according to procedures described in detail elsewhere (20, 21). Blood was withdrawn from each animal by orbital bleeding or from the jugular vein at the indicated time after immunization; serum was stored at –20°C until examined.

Measurement of OA-IgG_{2a} antibody levels. The radioimmunoassay for the measurement of ovalbumin-specific IgG_{2a} levels was performed as for measuring specific IgE levels (1). The quantity of IgG_{2a}-antibodies was expressed as a percentage of the total amount of radioactivity added. All the samples obtained in each ex-

periment were analysed during the same immunoassay run.

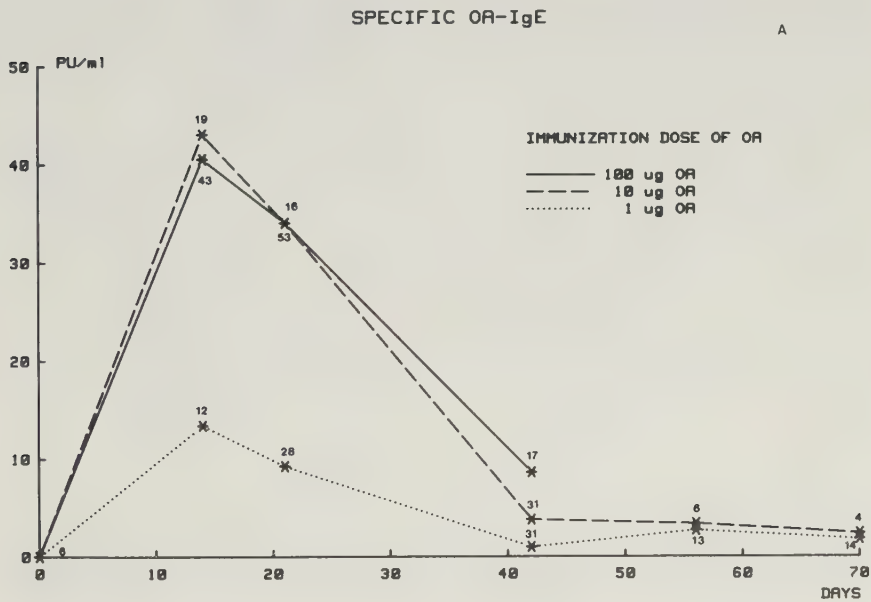
RESULTS

Serum levels of OA-IgE antibodies, total IgE, and the ratio of OA-IgE/total IgE as a function of OA dose and time after immunization

We previously reported in detail the pattern of temporal development of the response capacity in SD rats immunized i.p. with varying doses of OA and 100 mg $\text{Al}(\text{OH})_3$ with respect to bronchial anaphylactic reactivity (9). Fig. 1 in the present report shows the temporal development, in such animals, of OA-IgE antibody levels (frame A), of total IgE levels (frame B), and of the calculated ratio OA-IgE antibody to total IgE (frame C) in rats immunized with 1, 10, or 100 μg OA together with 100 mg $\text{Al}(\text{OH})_3$. The rats that provided the sera and whose numbers are included in these graphs, were those examined for bronchial anaphylactic

response capacity (9). It is clear from this figure that the pattern of development of the capacity to produce OA-IgE antibodies was similar in animals given 1 μg OA, 10 μg OA, or 100 μg OA, the magnitude of the response being significantly higher in the last two groups of animals than in the first. The maximum response of total IgE levels was observed on day 21 in all the groups; at that time, animals given 1 μg OA show the highest total IgE level.

The difference in total IgE levels at this time between the groups given 1 μg OA and 10 μg OA was significant ($P < 0.01$; Wilcoxon test). This led to the interesting observation that (Fig. 1C) the ratio OA-IgE/total IgE apparently followed a biphasic temporal pattern of development after injection of 1 μg and of 10 μg OA. Thus, this calculated ratio was almost two orders of magnitude higher on day 14 and day 56 than on day 42 for these two immunization regimens. However, this was not so in animals immunized with 100 μg OA.



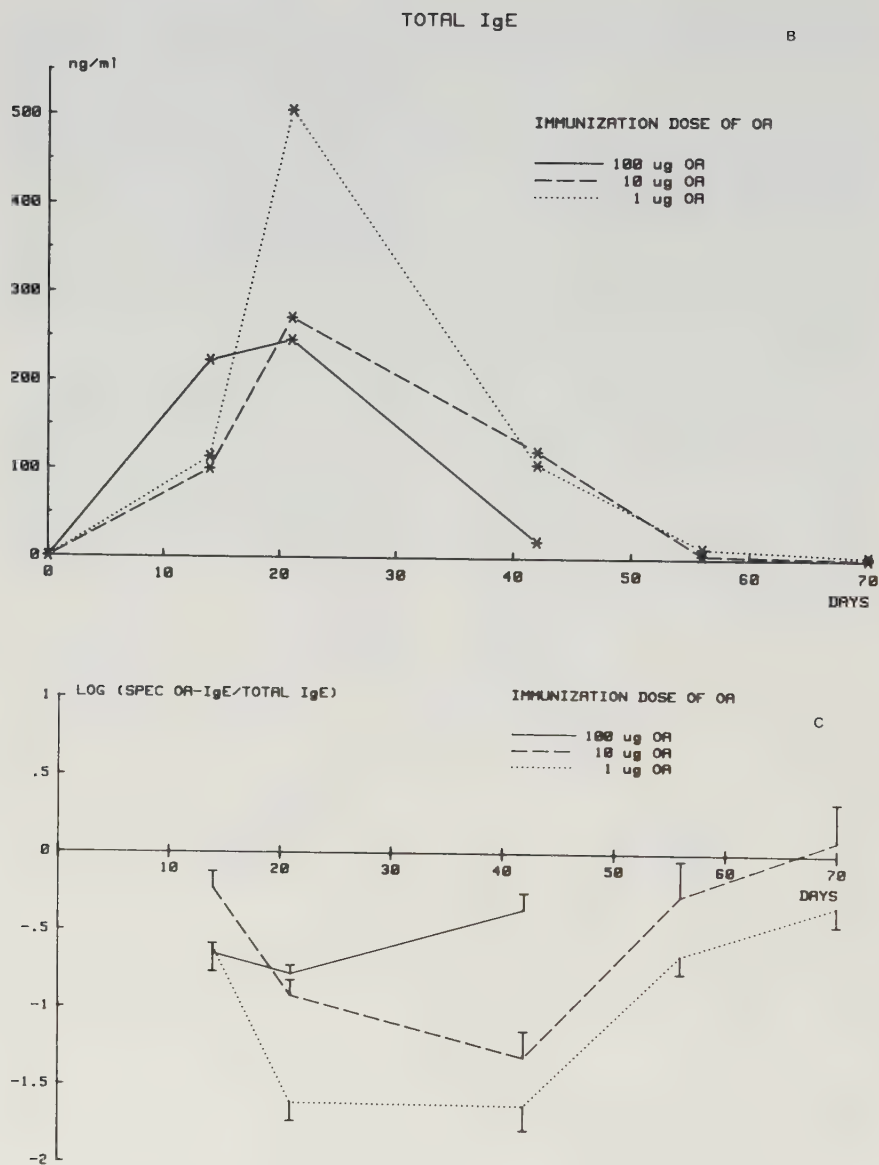


Fig. 1. Temporal development of levels of OA-IgE antibodies (A, previous page), total IgE (frame B), and log of ratio (R) of specific OA-IgE/total IgE (frame C) in SD rats immunized with 1, 10 or 100 µg OA together with 100 mg dried and resuspended $Al(OH)_3$. In frames A and B, each point represents the median value of the indicated number of animals; in frame C, the mean values $\pm$ SEM are given (ratios R are normally distributed).

Relation of serum levels of OA-IgE antibodies, total IgE, and the ratio OA-IgE/total IgE to anaphylactic reactivity

In Fig. 2, the median of the serum levels of OA-IgE antibodies and the mean of the ratio OA-IgE/total IgE of selected groups of animals are compared with the mean response of the pertinent groups with respect to bronchial anaphylactic reactivity *in vivo*. Fig. 2 represents the data collected from animals given 10 μ g OA in 100 mg Al(OH)₃. Note that the OA-IgE antibody levels and the ratio OA-IgE/total IgE decreased with time, whereas the bronchial

anaphylactic reactivity following a low challenge dose initially decreased slightly but then persisted unchanged, and that recorded after a high challenge dose increased with time.

Fig. 3 shows the data obtained with animals tested 42 days after immunization with 1, 10, or 100 μ g OA. Note that now the OA-IgE antibody levels and the ratio OA-IgE/total IgE increased with the immunization doses of OA, whereas the bronchial anaphylactic reactivity *in vivo* following a low, but not a high, challenge dose of OA seemed to decrease with increasing immunization doses of OA.

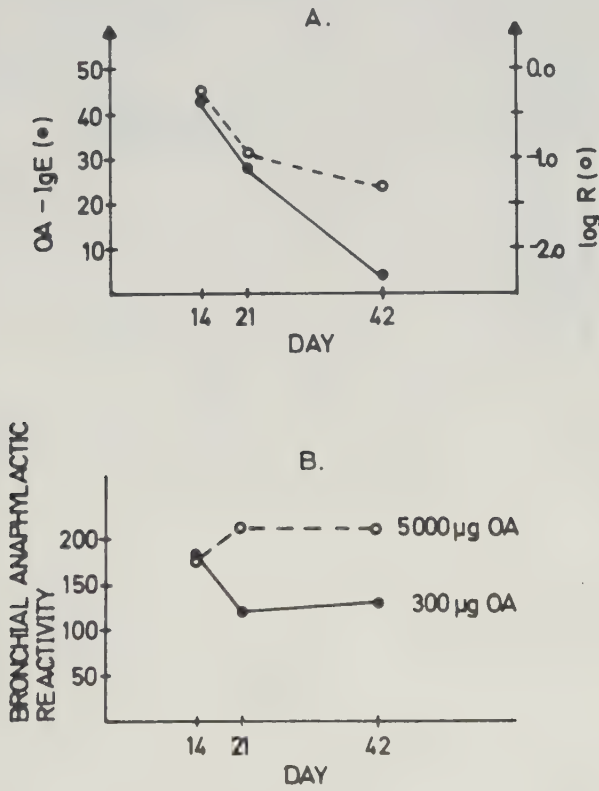


Fig. 2. A schematic representation of the temporal development of OA-IgE antibody levels (median values in PU/ml), and ratio R (OA-IgE/total IgE; mean values shown) (frame A; $n = 10-25$), and of bronchial anaphylactic reactivity (mean values, data from (9)) (frame B; $n = 44-50$) in SD rats immunized with 10 μ g OA together with 100 mg Al(OH)₃.

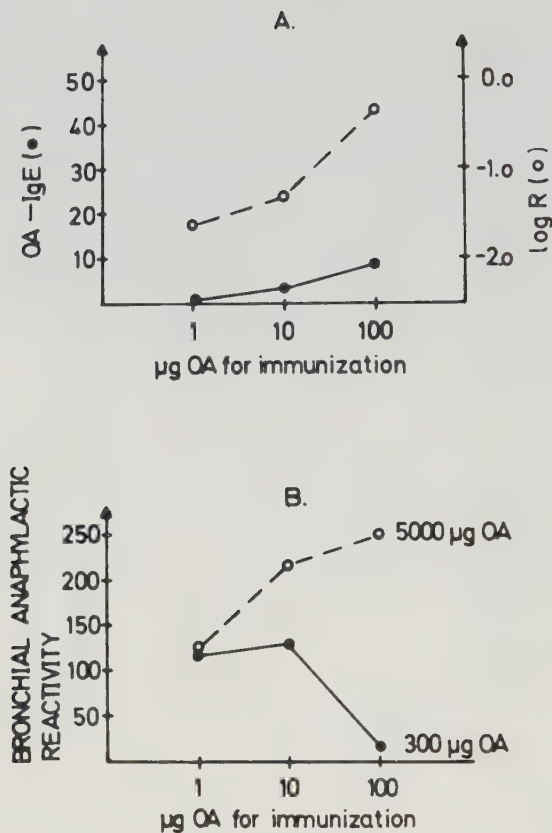


Fig. 3. A schematic representation of OA-IgE antibody levels (median values in PU/ml), and of ratio R (OA-IgE/total IgE; mean values shown) (frame A; $n = 17-31$), and of bronchial anaphylactic reactivity (mean values) (frame B; $n = 23-44$) in rats immunized with 1, 10, or 100 µg together with 100 mg $\text{Al}(\text{OH})_3$ 42 days before test.

Relation of serum levels of OA-IgE antibodies, total IgE, or ratio of OA-IgE/total IgE and bronchial anaphylactic reactivity in individual SD rats

Table 1 shows the correlations found between bronchial anaphylactic reactivity *in vivo* and serum levels of OA-IgE antibody, total IgE, or the ratio of OA-IgE antibody to total IgE in rats immunized with 1 µg, 10 µg, or 100 µg of OA 14, 21, or 42 days before the test. It is clear

that in some of the tests the possibility of a significant correlation between the serum level of OA-IgE antibody and reactivity cannot be excluded (e.g., animals tested 14 days after immunization with 10 µg OA), but most of the tests showed no sign of any correlation between reactivity and OA-IgE antibody titre, ratio OA-IgE to total IgE, or total IgE level. It is noteworthy that several animals immunized with 100 µg of OA and $\text{Al}(\text{OH})_3$ 42 days before the test showed significant levels of OA-IgE anti-

Table 1

Coefficients (r) of correlation between bronchial anaphylactic reactivity (in each animal recorded after a challenge dose of 300 μ g OA: $\Delta P\%$, or at a subsequent challenge with 5000 μ g OA: ΔP max %) and specific OA-IgE antibody level, total IgE, or ratio R defined as specific OA-IgE antibody/total IgE

		Immunization dose and time of test								
		1 μ g OA			10 μ g OA			100 μ OA		
		14 days	21 days	42 days	14 days	21 days	42 days	14 days	21 days	42 days
Spec. OA-IgE vs										
$\Delta P\%$		-0.44 (12)	-0.21 (10)	0.13 (13)	0.59** (18)	0.16 (11)	0.56 (11)	0.05 (30)	0.13 (31)	-0.17 (17)
ΔP max %		0.42 (12)	0.45 (10)	0.16 (13)	0.26 (18)	-0.42 (11)	0.64* (11)	0.03 (27)	0.44** (29)	0.82*** (17)
Tot. IgE vs										
$\Delta P\%$		-0.20 (12)	0.02 (10)	0.33 (13)	0.20 (18)	0.04 (11)	0.29 (11)	-0.05 (30)	0.17 (31)	0.05 (17)
ΔP max %		0.45 (12)	-0.32 (10)	0.13 (13)	0.04 (18)	-0.19 (11)	0.62* (11)	-0.40* (27)	-0.07 (29)	0.31 (17)
Log $\frac{\text{Spec. OA-IgE}}{\text{Tot. IgE}}$ vs										
$\Delta P\%$		-0.52 (12)	0.02 (10)	-0.08 (13)	0.48* (18)	0.09 (11)	-0.55 (11)	0.10 (30)	-0.07 (31)	-0.26 (17)
ΔP max %		0.31 (12)	0.66* (10)	0.32 (13)	0.10 (18)	-0.22 (11)	-0.77** (11)	0.26 (27)	0.41* (29)	0.37 (17)
Spec. OA-IgE vs Tot. IgE										
		0.76** (12)	0.51** (28)	0.26 (31)	0.59** (19)	0.43 (16)	-0.22 (31)	0.33* (43)	0.37* (53)	0.36 (17)

Figures in brackets denote the number of animals.

Probability levels *0.01 < P < 0.05; **0.001 < P < 0.01; *** P < 0.001.

bodies (the figures registered in a group of six animals were 29, 20, 17, 14, 7.5 and 3.7 PU/ml) yet did not react to a challenge dose of 300 μ g OA. However, when the provocation dose of OA in these animals was increased to 5 mg, a highly significant correlation was found between OA-IgE antibody titre and reactivity. This was not the case in animals given 10 μ g OA 14 or 21 days before test. In general, the correlation between the bronchial anaphylactic reactivity and antibody level was not increased by substituting the ratio of OA-IgE antibodies to total IgE for the OA-IgE. It is also noteworthy

that the present data showed a convincing correlation between OA-IgE antibody levels and total IgE level in animals immunized with 1 μ g OA but not in those given 100 μ g OA.

*Relation between serum level of
OA-IgG_{2a} antibodies and bronchial anaphylactic
reactivity in vivo*

Table 2 shows that the bronchial anaphylactic response capacity *in vivo* is not correlated to serum levels of specific IgG_{2a} antibody. Animals injected with various doses of OA in FCA

Table 2

Bronchial anaphylactic reactivity (BAR) (mean $\pm$ SEM) of % increase in intratracheal pressure following a challenge dose of 300 μ g OA (Δ P%) or a subsequent challenge with 5000 μ g OA (Δ P max %) and specific OA-IgG_{2a} serum antibody levels (median) in selected groups of animals immunized as indicated

Immunization regimen	Day(s) for test	BAR		OA-IgE PU/ml	IgG _{2a} (% of total radioactivity)	(n)
		Δ P %	Δ P max %			
10 μ g OA + FCA	14	49 $\pm$ 20	205 $\pm$ 10	6.5	39	(6)
100 μ g OA + FCA	15	20 $\pm$ 3	203 $\pm$ 8	7.5	59	(6)
10 mg OA + FCA	14	29 $\pm$ 5	200 $\pm$ 31	1.4	54	(6)
10 μ g OA + Al (OH) ₃	41-43	177 $\pm$ 23	203 $\pm$ 13	7	25	(11)

Figures in brackets denote the number of animals in each group.

showed a poor bronchial anaphylactic response capacity (at a low challenge dose of OA) but high OA-IgG_{2a} antibody levels; those given a low immunization dose of OA together with Al(OH)₃ reacted vigorously to the same challenge dose despite low OA-IgG_{2a} antibody levels.

DISCUSSION

Our investigation was concerned with the relation, in rats, between serum levels of specific IgE- and IgG_{2a} antibodies, on the one hand, and bronchial anaphylactic response capacity *in vivo*, on the other. A number of previously published studies have failed to demonstrate a simple correlation between serum IgE antibody titres, histamine release from a variety of tissues *in vitro* and/or antigen-induced anaphylactic reactivity *in vivo* (e.g. 5, 6, 24, 29). With this in mind, it is clearly noteworthy that a highly significant correlation between serum OA-IgE antibody levels and bronchial anaphylactic reactivity, precipitated by a high dose of antigen, was in fact recorded by us in animals immunized with 100 μ g OA 42 days before test, although the serum OA-IgE antibody titres at that point of time were already declining. A correlation, though less close, was also found between bronchial anaphylaxis (precipitated by a low antigen dose) and OA-IgE antibody levels in animals given 10 μ g OA 14 days before the test. However, under other very similar experimental

conditions no correlation was observed, despite high serum levels of OA-IgE antibody and considerable bronchial anaphylactic reactivity. How can this difference be explained?

Possible general explanations should first be considered to account for the lack of correlation between response capacity and OA-IgE antibody level under certain experimental conditions.

Firstly, Conroy et al. (7), who used basophilic leukocytes, observed that only a small number of cell-fixed IgE antibodies were necessary to prepare these cells for histamine release. Furthermore, cells from individuals having approximately the same number of IgE varied widely in their capacity to release histamine when challenged with anti-IgE. The authors concluded that an intrinsic property of human basophils, which they referred to as "releasability", is an important parameter in determining mediator release and that this releasability differs from individual to individual. However, available data are not yet sufficient to establish that such a parameter is important in the determination of the degree of rat mast cell histamine release *in vitro*.

Secondly, many factors may interfere with mediator release and that this releasability differs from individual to individual. However, and mediators of inflammation (3, 25). It is also well known that histamine release from rat peritoneal mast cells *in vitro*, induced by IgE-dependent secretagogues, is markedly en-

hanced by certain phospholipids, e.g., phosphatidyl serine (16) and that on incubation *in vitro* with human basophils certain viruses enhance ragweed antigen E or anti-IgE induced histamine release (11).

Thirdly, some normal individuals with specific IgE antibodies, and manifesting antigen-induced histamine release from basophilic leukocytes, do not exhibit any manifestation of disease on natural exposure to allergen (19). Furthermore, there is no correlation between severity of disease and serum level of specific IgE antibody (4, 26); especially in asthmatics, the mild immediate type of allergen-induced bronchospasm seems to depend more on non-specific bronchial hyperreactivity than on the level of circulating specific IgE antibody (26). In rats bronchial reactivity to administered exogenous serotonin varies from strain to strain (22).

Fourthly, in our investigation antigen was administered intravenously, preliminary experiments having indicated that, in our hands, even high doses (10 mg/ml) of OA administered as an aerosol failed to precipitate a response. Antigen given i.v. may have induced changes in lung mechanics through mediators released from extrapulmonary organs and have reached the lung through the circulation.

It is clear that all such mechanisms might conceivably contribute to a weak correlation between degree of release and antibody level. However, the existence of a highly significant positive correlation between OA-IgE serum antibody levels and response capacity under some, but not all, of the conditions studied suggests that nonspecific factors such as releasability, endogenous hormones, degree of bronchial reactivity and route of antigen administration are of little value in the search for an explanation of the lack of correlation under certain circumstances. This we consider a crucial point.

It would rather seem that the lack of a general correlation between serum antibody level and bronchial anaphylactic response capacity has an immunological basis. Firstly, is a correlation masked by the longer half-life of tissue-bound IgE compared with serum IgE? Sydbom & Karlsson (30) observed a significant, although

not convincing, correlation between peritoneal mast cell response capacity and OA-IgE antibody level on day 11 after immunization, but not later. However, in our experiments, serum levels of OA-IgE were still high 3 weeks after immunization with both 1 and 10 μ g OA, indicating that any such masking cannot be the major reason for the lack of correlation at that point in time. Furthermore, serum levels of OA-IgE antibody were lower in the animals immunized 42 days before the test with 100 μ g OA (those showing a highly significant correlation) than in most groups of animals examined 14 or 21 days after immunization.

Secondly, is the correlation dependent on the level of nonspecific IgE? In the human basophilic leukocyte system, Zeiss et al. (33) found no correlation between the level of allergen-specific IgE in plasma and the number of molecules of allergen-specific IgE per basophil, but rather a correlation between the ratio of allergen specific IgE to total serum IgE and the last-mentioned variable. Cell reactivity, i.e., the highest attainable degree of histamine release at any antigen concentration, was dependent on the number of allergen-specific IgE molecules per basophil, but cell sensitivity, i.e., the lowest antigen concentration inducing a defined degree of histamine release, was not. However, in the present system, we did not find a better correlation with bronchial anaphylactic response to a low or to a high antigen provocation dose when the ratio OA-IgE antibody to total IgE was substituted for the OA-IgE antibody level. Thus, from data published by Jarrett et al. (13), who showed that active anaphylactic reactions in rats are not influenced by *Nippostrongylus brasiliensis*-induced increases in non-specific IgE; and from the present data, it seems that the effect of total IgE is not sufficient to explain the above-mentioned lack of correlation.

Thirdly, Jarrett & Stewart (12) suggested that detectable levels of IgE antibodies might not appear in serum until IgE fixing sites in the pertinent tissues have been saturated, i.e., fixation could conceivably withdraw OA-IgE antibodies from the circulation. However, in our experiments, animals immunized with 10 or

100 µg OA showed high serum levels of OA-IgE antibodies but there was still no convincing correlation between OA-IgE titre and bronchial anaphylactic reaction to a low challenge dose in any of these groups, except in those mentioned above.

Fourthly, *in vivo* elimination by blocking non-reaginic antibody of the challenge dose of antigen from the circulation could conceivably mask a correlation. Blocking antibody could have another profile of production or avidity maturation than reaginic antibody. However, in the present experiments this is not a likely explanation because even following a low challenge dose of antigen, there is a sufficient amount of it present to precipitate a vigorous physiological response (see (9)). Furthermore, increasing the challenge dose more than tenfold did not increase the degree of correlation (except for animals immunized with 100 µg OA 42 days before test).

Fifthly, a protein antigen and an outbred rat strain were used in our experiments. It is not clear to what extent the outcome of the experiments is influenced by the effect of genetic polymorphism on clonotypic expression of IgE and IgG_{2a}, average affinity of antibodies present and degree of heterogeneity of antigenic determinants on OA.

We previously showed (9) that animals immunized with 10 µg OA and 100 mg Al(OH)₃ tended to develop a bronchial anaphylactic response capacity, as revealed by a low challenge dose of OA according to a "biphasic" pattern; a high degree of reactivity on day 14 is followed by a lower degree of reactivity which is of similar magnitude on day 21 and 42 (Fig. 3). A qualitative difference in the antibodies mediating the two parts of the temporal response development curve is suggested by the finding that some anti-allergic agents are able to inhibit the bronchial anaphylactic reactivity late, but not early, after immunization (9). A "biphasic" pattern of reaginic antibody synthesis is apparently not a novel observation, since data published by Petillo & Smith (23) (Table 1) and by Jarrett et al. (14) may also be interpreted as showing the existence of a "biphasic" time course of the appearance of reaginic antibodies

in the serum of rats immunized with alum precipitated OA. In our hands, in the early part of the bronchial anaphylactic response capacity curve, the reactivity of rats immunized with 10 µg OA showed some degree of correlation with the OA-IgE antibody levels, indicating that IgE-antibodies could, at least partly, mediate the response at this time. No correlation was demonstrable between reactivity and OA-IgE antibody levels in animals tested during the latter part of the temporal response curve, or in the group of animals immunized with only 1 µg OA. But it is not known why. In this context, it should be pointed out that Church (6) reported that both the severity of an active anaphylactic bronchoconstriction and the level of circulating reaginic antibodies (PCA test) in rats sensitized with *Nippostrongylus brasiliensis* did not reach a maximum until 5 weeks after the injection. As pointed out by Church (6), these data deviate from those of Jarrett & Stewart (12) on the temporal development of the active cutaneous anaphylactic reaction; the last-mentioned data conform to the temporal profile of IgE antibody synthesis in the present report.

Thus, the possibilities of several homocytotropic antibodies contributing to the response cannot be dismissed. In this context the results of Yamamoto et al. (32) deserve consideration. These authors found that within individual animals the relative degrees of antigen-induced histamine release from actively sensitized lung tissue, mesenteric tissue, and skin were not correlated to each other or to the level of circulating antigen-specific reagent. Our experiments (2) showed that the development of lung tissue response capacity (estimated by antigen-induced release of histamine *in vitro*) can apparently be qualitatively and temporally differentiated from that of serosal mast cells.

The short-term sensitizing homocytotropic IgG_{2a} antibody (for review see 28) is a conceivable candidate for mediating at least part of the bronchial anaphylactic responses either 14–21 days or 6 weeks after immunization. However, the role played by this kind of antibody can be questioned. Firstly, we examined rats immunized with various amounts of OA in Freund's complete adjuvant. This regimen is known to

induce high levels of OA-IgG_{2a} antibodies, which was confirmed. However, such animals showed no more than a weak bronchial anaphylactic response capacity following a low challenge dose of OA.

Secondly, we found no bronchial anaphylactic responses to a challenge dose of 300 µg of OA 42 days after immunization when the immunization dose of OA was increased from 10 to 100 µg OA (Fig. 2). There is only little information available about the dependence of the level or the pattern of development and persistence of IgG_{2a} antibody responses in rats on the antigen dose used at immunization. However, like the synthesis of mouse IgG₁ antibody (18) one would hardly expect that reactivity due to IgG_{2a} antibody activity would decrease with increasing immunization doses. However, such a phenomenon is well known in IgE antibody synthesis.

It must be stressed that we have not yet succeeded in consistently transferring responses with serum passively. Thus, unfortunately, we cannot clearly state whether the antibodies mediating the anaphylactic responses are thermolabile. Characterization of the homocytotropic antibodies responsible for the bronchial anaphylactic reactivities at various points in time obviously requires further research.

Together with a number of other reports our observations in rats suggest that more than one type of reaginic antibody might be responsible for sensitization of lung tissue for anaphylactic reactivity. We have previously reported data indicating a dissociation in the development of lung and tracheal tissue response capacity in SD rats (2) with respect to histamine release from chopped tissue *in vitro*. Lehrer & Bazin (17) recently reported that rat IgE myeloma proteins are heterogeneous in PCA inhibitory capacity. Also in humans the existence of IgE-antibody subclasses has been considered on the basis of a differential capacity to passively sensitize lung tissue and basophils (8, 10). Our peculiar observation that the ratio OA-IgE/total IgE in animals given 1 µg or 10 µg OA follows a biphasic temporal pattern of development (Fig. 1C) could be interpreted as strengthening the possibility of subclasses of IgE with different kinetics of production.

In conclusion, our data indicate that lung tissue response capacity *in vivo* is correlated to OA-IgE antibody levels provided the experimental conditions are carefully controlled. However, no correlation between tissue reactivity *in vivo* and serum OA-IgE antibody level is demonstrable under a number of other experimental conditions. The reason for this is not clear, but one possible explanation is the existence of several reaginic antibody subclasses contributing to the response.

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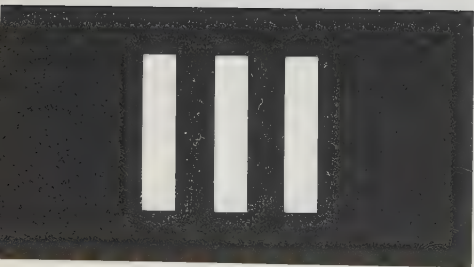
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The Non-Specific Enhancement of Allergy

III. Precipitation of Bronchial Anaphylactic Reactivity in Primed Rats by Injection of Alum or *B. pertussis* Vaccine: Relation of Response Capacity to IgE and IgG_{2a} Antibody Levels

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SD rats were sensitized by an i.p. injection of 1 µg or 10 µg ovalbumin (OA) together with 10 mg Silica gel. At the indicated time after sensitization, allergic response capacity of the animals was estimated by measuring changes in intratracheal pressure induced by an i.v. injection of 0.3 mg OA (low challenge dose) or 5 mg OA (high challenge dose). Animals given 1 µg OA in Silica gel showed no response capacity at 14, 35 or 47–48 days after sensitization. Animals injected with 10 µg OA showed a clear-cut response with a peak at 14 days; at 7 weeks after immunization the response capacity had faded almost completely. Specific OA-IgE antibody and total IgE serum levels were examined by radioimmunoassay. A slight increase in OA-IgE antibody was recorded in animals injected 14 days before test with 10 µg ovalbumin and Silica gel; animals given 1 µg OA and Silica showed no OA-IgE antibody.

Five weeks after sensitization, some animals of each group were injected i.p. with 100 mg alum, *B. pertussis* vaccine (2 ml of Perthydrat®), or saline, all injections being made without any further antigen addition. 12–13 days after such an injection, alum-treated animals showed high response capacity at bronchial anaphylactic tests and high OA-IgE antibody levels but comparably low levels of total IgE. Animals injected with *B. pertussis*, on the other hand, showed low response capacity (bronchial anaphylactic tests and OA-IgE antibody levels) but high total IgE-antibody levels. The bronchial anaphylactic response in the *B. pertussis*-injected animals but not the alum-injected animals was significantly correlated to serum IgG_{2a} antibody levels.

These results show that injection of alum or *B. pertussis* vaccine without antigen can precipitate/enhance anaphylactic response capacity and production of specific and non-specific IgE and IgG_{2a}.

Key words: adjuvant: silica; alum; *B. pertussis*; antigen: ovalbumin; bronchial anaphylaxis; IgE and IgG_{2a} antibody levels; SD rats.

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CLINICAL ASPECTS

The triggering mechanisms of human IgE-mediated allergy are still largely unknown; however, viral infection is a likely candidate. It has become apparent that IgE antibody synthesis as well as total IgE synthesis in experimental animals can be non-specifically potentiated by various means, e.g. by parasitic infection, by treatment with mercuric chloride or by injections of *B. pertussis* organisms. In the present paper we report the results of experiments designed on the rat to examine whether treatment with IgE antibody synthesis potentiating agents, such as alum and *B. pertussis* vaccine, enhances/induces the capacity in primed animals to respond to

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antigen challenge with bronchial anaphylactic reactions *in vivo*. The results show that injection of alum or *B. pertussis* vaccine without antigen can precipitate/enhance production of specific and non-specific IgE and IgG_{2a} antibodies and anaphylactic response capacity even in animals which do not respond after the priming injection.

In recent years it has become apparent that IgE antibody synthesis as well as total IgE synthesis in experimental animals can be non-specifically potentiated by various means, e.g., by parasitic infection (5, 6), by treatment with mercuric chloride (11) or by injections of *B. pertussis* (Bp) organisms (10, 12). The potentiating effect of the parasite commonly employed in rats, *Nippostrongylus brasiliensis*, can be observed if infection with the nematode takes place approximately 11 days after immunization but not earlier. HgCl₂ potentiated the IgE response whether injections were begun on day 0 or day 11 after immunization; potentiation was observed in the high responder strain BN but not in the low responder Lewis strain.

In an accompanying report (2) we show that IgE antibody synthesis and anaphylactic response capacity (in the form of antigen-induced histamine release from serosal mast cells and from lung tissue at challenge *in vitro*) can be "precipitated" in PVG and Sprague Dawley (SD) rats, which have been primed with antigen but do not manifest such response capacity with injection of alum alone.

In the present paper we report the results of experiments designed to examine whether treatment with IgE antibody synthesis potentiating agents, such as alum and Bp vaccine, induces/enhances the capacity in primed animals to respond to antigen challenge with bronchial anaphylactic reactions *in vivo*.

EXPERIMENTAL PROCEDURE

Animals and immunization procedure

The animals used were outbred male Sprague Dawley rats obtained in specific pathogen-free (SPF) status at a weight of about 200 g from Møllegaards Avlscentrum, Ejby, Skensved, Denmark. At our laboratory the animals were reared under conventional animal housing conditions.

Sensitization was performed by intraperitoneal injections of either 1 µg or 10 µg ovalbumin (OA) (Sigma grade III, crystallised and lyophilised) in 0.5 ml saline containing 10 mg of amorphous Silica gel (Aerosil, particle diameter 25–50 nm, Degussa, Frankfurt am Main, obtained via Matak, Malmö, Sweden) or by 1 µg OA in two ampoules of Perthrydal® (Institute Pasteur Production, Paris; each ampoule contains in 1 ml, 5×10^9 organisms of *B. pertussis* and 1.25 mg Al(OH)₃). On day 35 after immunization, groups of animals were given 100 mg of dried Al(OH)₃ (F 2200 Reheis Chemical Company, obtained through AB Astra, Södertälje) resuspended in 0.5 ml saline, or saline. A few groups of animals were immunized according to other regimens as specified in section V under Results.

Respiratory measurements

The animals were tested at the indicated time after immunization. They were anaesthetised with 40 mg/kg i.p. pentobarbital (Mebumal®, ACO, Sweden) and 25 mg/kg metomidate chloride (Hypnodil®, Leo, Sweden); succinylcholine chloride (Celocurin®, Vitrum, Sweden), 5 mg/kg, was administered i.p. to prevent spontaneous respiration. Respiratory measurements were performed essentially as described by Stotland & Share (13) as reported in detail previously (3).

In brief, the trachea was cannulated and the rat ventilated with a Braun constant respiratory pump in a closed system. Tracheal pressure was recorded through a side arm of the cannula connected to a Statham P23AC pressure transducer. Respiratory rate was set at 70 strokes/min with a tracheal pressure of 7 cm H₂O (1 cm H₂O = 98 N/m²) (tidal volume range 2.0–3.75 ml). Mean arterial blood pressure was recorded by a Statham P23AC pressure transducer throughout the entire experiment through a catheter inserted in the right a. carotis. The

blood pressure pulses were transmitted to a tachogram, where the heart rate was recorded. All recordings were made on a Grass polygraph model 7B. Animals were challenged intravenously through a catheter inserted in the right jugular vein. A low antigen dose (0.3 mg OA) was injected and the response recorded. Approximately 2 min later an additional high dose (5 mg OA) was injected and the response again recorded. The resultant increase in tracheal pressure, termed active bronchial anaphylaxis, was used as a measure of bronchoconstriction.

OA-IgE antibody and total serum IgE levels were measured according to procedures described in detail elsewhere (7, 8). Blood was withdrawn from each animal by orbital bleeding or from the jugular vein at the indicated time after immunization; serum was stored at -20°C until examined.

Measurement of OA-IgG_{2a} antibody levels

The radioimmunoassay for the measurement of ovalbumin-specific IgG_{2a} levels was performed as for measuring specific IgE levels (details in ref. 1).

Before log transformation, figures for antibody levels lower than 1 PU/ml, or total IgE lower than 1 ng/ml, were arbitrarily put at 1 PU/ml and 1 ng/ml, respectively.

Statistics

The figures recorded for bronchial anaphylactic response were found to be normally distributed when a sufficient number of experiments were performed. Mean values and standard error of the mean were used for graphical illustration.

RESULTS

I. Development of response capacity in rats immunized with 1 μg or 10 μg together with Silica gel or Perthrydal

Groups of rats were injected i.p. with either 1

μg or 10 μg OA together with 10 mg Silica gel. The animals were tested 7, 14, 35, or 47–48 days after sensitization. Bronchial anaphylactic responses were recorded after challenge i.v. with 0.3 mg OA and after a further injection of 5 mg OA as described under Experimental procedure. As shown in Fig. 1A, animals immunized with 1 μg OA together with Silica gel uniformly failed to respond to either of the two provocation doses of OA at any of the examination time points after immunization. On the other hand, animals immunized with 10 μg OA together with Silica gel responded well to both the low and the high provocation doses of OA at day 14 after immunization; the response capacity of animals immunized in this way had decreased at test days 35 or 47–48 after immunization. There was a highly significant correlation between the responses recorded with the low and those recorded with the high provocation dose under these experimental conditions.

Another group of rats was immunized with 1 μg OA together with two ampoules of Perthrydal. These animals were challenged 1, 2, 3, 5, or 7 weeks after sensitization. Fig. 1B shows that until day 35 the bronchial response capacity increased with time with a highly significant correlation between response to low and to high challenge doses of OA.

II. Effects of i.p. injections of alum in primed rats

Groups of rats were immunized i.p. with 1 μg or 10 μg OA together with 10 mg Silica gel or two ampoules of Perthrydal as described above. Five weeks later, the animals received either 100 mg $\text{Al}(\text{OH})_3$, two ampoules of Perthrydal, or saline i.p. Response capacity of these animals was determined at days 47–48 after the initial OA injection. As shown in Fig. 2A, injection of alum in rats sensitized to OA together with Silica gel led to development of a marked response capacity even in animals not showing anaphylactic reactivity initially (i.e., those sensitized with 1 μg OA + 10 mg Silica). Injection of Perthrydal also precipitated/enhanced response capacity, although less markedly than the injection of alum. Note that the magnitude of response

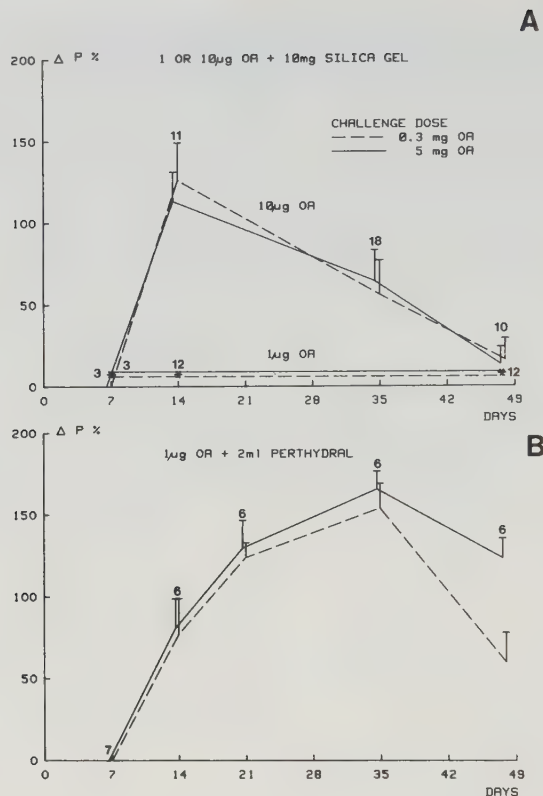


Fig. 1. Bronchial anaphylactic responses of rats immunized on day 0 with 1 µg or 10 µg OA together with 10 mg Silica gel i.p. (Frame A) or 2 ml Perthydral (Frame B). Challenge was performed on days 7, 14, 35 and 47–48 after immunization with a low (0.3 mg OA) and a high (5 mg OA) challenge dose of OA i.v. Response capacity ($\Delta P\%$) was expressed as per cent increase of intratracheal pressure recorded as described in Experimental procedure. Figures given denote number of experiments; bars denote SEM.

capacity induced by injection of alum or Perthydral did not differ between rats given 1 µg or 10 µg OA together with Silica gel at the primary immunization. Fig. 2B shows that an alum injection at day 35 of animals primed with 1 µg OA together with two ampoules of Perthydral (if anything) leads to reduced response capacity at day 47–48. It should be noted that these animals before alum injection show almost maximal response capacity (Fig. 1B).

III. Serum levels of OA-IgE and OA-IgG_{2a} antibodies and total IgE after primary sensitization and following precipitation/enhancement of response capacity by injection of alum or Perthydral

Fig. 3 shows the temporal development of specific OA-IgE (frame A), specific OA-IgG_{2a} (frame B) antibody levels and of total IgE levels (frame C), and of the calculated ratio

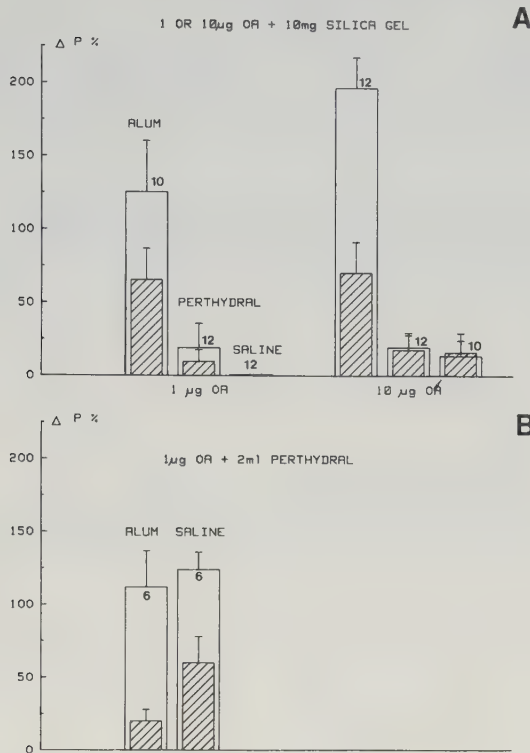
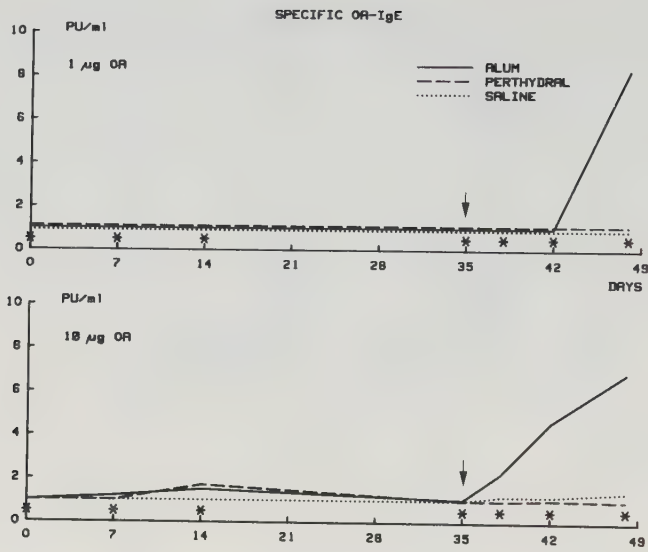


Fig. 2. Bronchial anaphylactic responses of rats immunized on day 0 with 1 μ g or 10 μ g OA together with 10 mg Silica gel (Frame A) or 1 μ g OA together with 2 ampoules of Perthydral (Frame B). Animals were reinjected with either 100 mg of alum, 2 ampoules of Perthydral or saline on day 35 and tests were performed on day 47–48 as described in legend to Fig. 1. Hatched bars denote mean of responses to a low challenge dose of OA (0.3 mg), open bars denote mean of responses to a high dose of OA (5 mg). Figures given denote the number of experiments; bars denote SEM.

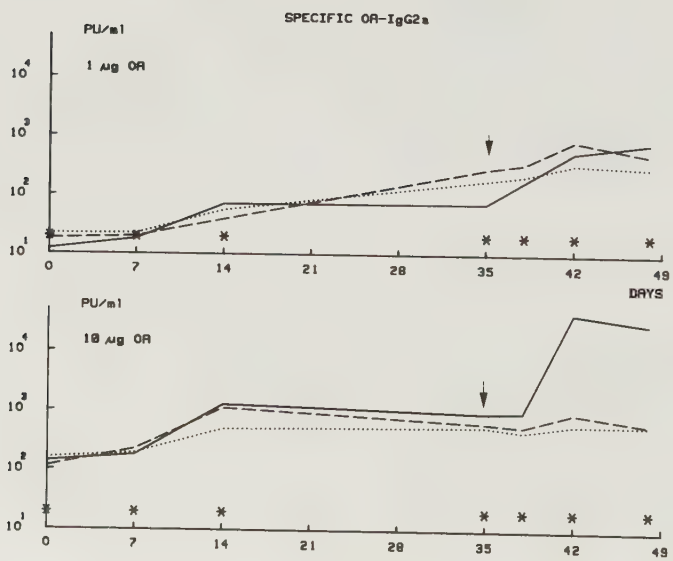
specific OA-IgE to total IgE (frame D) in animals immunized with 1 μ g or 10 μ g OA together with 10 mg Silica gel day 0 and injected with 100 mg $\text{Al}(\text{OH})_3$ or two ampoules of Perthydral day 35. Clearly, $\text{Al}(\text{OH})_3$ is much more effective than Perthydral in enhancing OA-IgE antibody synthesis; on the other hand, Perthydral more effectively than $\text{Al}(\text{OH})_3$, enhances total IgE synthesis. Consequently, the ratio specific OA-IgE to total IgE decreases much more after Perthydral administration than after alum injection (frame D). OA-IgC_{2a} antibody levels increased following primary in-

jection of either 1 μ g or 10 μ g OA together with Silica gel; animals given 10 μ g OA throughout showing approximately a 10-fold higher antibody level than those given 1 μ g OA. In animals primarily given 10 μ g OA, injection with alum alone at day 35 increased OA-IgC_{2a} antibody levels more than did injection of Perthydral.

Fig. 4 shows that in animals primed with 1 μ g OA and two ampoules of Perthydral, a booster injection of alum leads to highly increased total IgE levels but does not significantly affect OA-IgE levels.



A



B

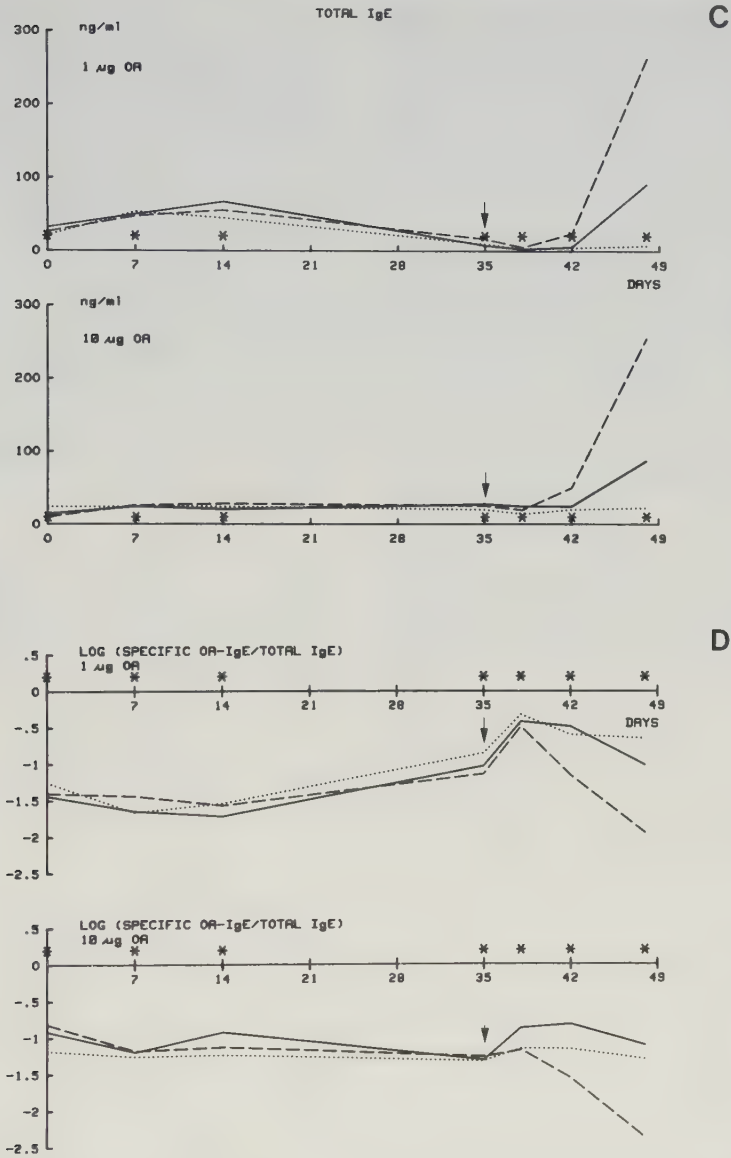


Fig. 3. Development of serum OA-IgE antibody (Frame A), OA-IgG_{2a} antibody (Frame B), total IgE (Frame C), and log ratio (OA-IgE to total IgE) (Frame D) levels in rats immunized as indicated with 1 µg or 10 µg OA together with 10 mg Silica gel day 0 and reinjected with saline, 100 mg of alum, or 2 ml Perthrydral day 35 (indicated by arrows). Each curve represents median values of six animals at each indicated time point (asterisk).

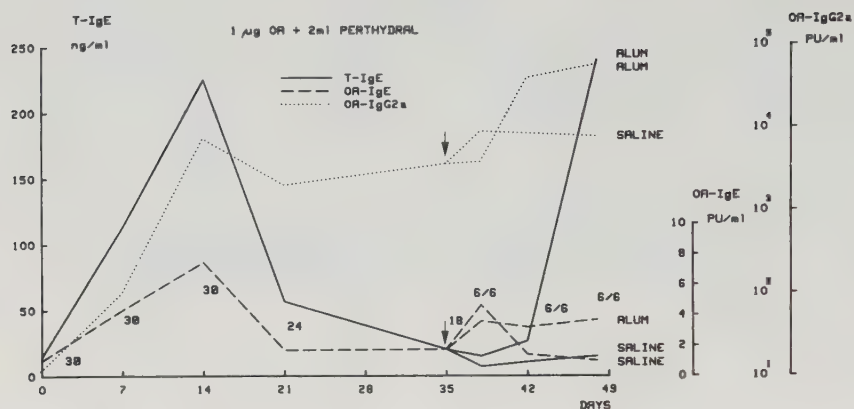


Fig. 4. Development of serum total IgE, OA-IgE and OA-IgG_{2a} antibody levels (as denoted in figure) in rats immunized with 1 µg OA together with 2 ampoules of Perthrydal and reinjected with 100 mg alum or saline day 35 (arrow). The figures denote the total number of experimental animals used.

Table 1

Correlation coefficients (*r*) recorded at examination of linear correlation between bronchial anaphylactic reactivity (in each animal recorded at a challenge dose of 0.3 mg OA: Δ*P*% or at a subsequent challenge with 5 mg OA: Δ*P*max%) and log specific OA-IgE (PU/ml) or log OA-IgG_{2a} (PU/ml) antibody levels, log total IgE (ng/ml) or log ratio *R* defined as specific OA-IgE to total IgE. Tests were performed day 47–48 after priming the animals with 1 or 10 µg OA together with Silica gel, i.e., 12–13 days after injection of alum or Perthrydal

	Immunization regimen			
	1 µg OA + 10 mg Silica gel		10 µg OA + 10 mg Silica gel	
	Al(OH) ₃	Perthrydal	Al(OH) ₃	Perthrydal
log Spec. OA-IgE vs.				
Δ <i>P</i> %	0.82**	0.47	0.08	0.27
Δ <i>P</i> max %	0.37	0.47	0.50*	0.25
log Total IgE vs.				
Δ <i>P</i> %	0.21	-0.60*	0.17	-0.12
Δ <i>P</i> max %	-0.60*	-0.60*	0.07	-0.18
log <i>R</i> vs.				
Δ <i>P</i> %	0.59*	0.63**	-0.04	0.22
Δ <i>P</i> max %	0.88***	0.63**	0.64**	0.28
log Spec. OA-IgG _{2a} vs.				
Δ <i>P</i> %	0.27	0.87***	-0.45	0.55*
Δ <i>P</i> max %	0.56*	0.87***	0.67**	0.50*
	<i>n</i> = 10	<i>n</i> = 12	<i>n</i> = 12	<i>n</i> = 12

n = number of animals.

Probability levels: *0.01 < *P* < 0.05; **0.001 < *P* < 0.01; ****P* < 0.001.

Table 2

Correlation coefficients (r) recorded at examination of linear correlation between bronchial anaphylactic reactivity (in each animal recorded at a challenge dose of 0.3 mg OA: $\Delta P\%$ or at a subsequent challenge with 5 mg OA: $\Delta P_{\max}\%$ in animals primed with 1 μg OA and 2 ampoules of Perthrydal) and log specific OA-IgE or log OA-IgG_{2a} antibody levels or log total IgE, or log ratio R defined as specific OA-IgE to total IgE. Tests were performed day 48 after priming of the animals with 1 μg OA and Perthrydal, i.e., 12 days after injection of alum or saline

	Booster injection	
	Al(OH) ₃	Saline
log Spec. OA-IgE vs.		
$\Delta P\%$	0.83*	0.14
$\Delta P_{\max}\%$	0.98***	0.05
log Total IgE vs.		
$\Delta P\%$	0.20	-0.85**
$\Delta P_{\max}\%$	0.39	0.23
log R vs.		
$\Delta P\%$	0.76*	0.79*
$\Delta P_{\max}\%$	0.79*	0.22
log Spec. OA-IgG _{2a} vs.		
$\Delta P\%$	0.77*	-0.61
$\Delta P_{\max}\%$	0.96***	0.1
	n = 6	n = 6

n = number of animals.

Probability levels: *0.01 < P < 0.05; **0.001 < P < 0.01;

***P < 0.001.

IV. Relation between serum levels of specific OA-IgE antibody, total IgE, ratio of OA-IgE to total IgE, or specific OA-IgG_{2a} and bronchial anaphylactic response capacity in adjuvant-treated animals

Table 1 shows the correlation observed between bronchial anaphylactic reactivity *in vivo* and serum levels of log OA-IgE antibody, log total IgE, the log ratio of OA-IgE to total IgE, and log OA-IgG_{2a} in animals immunized with 1 μg OA or with 10 μg OA in 10 mg Silica, injected with alum or Perthrydal day 35 and tested 12–13 days later. Note that for animals given 1 μg OA, but not for those given 10 μg OA, together with Silica gel and then injected with alum,

there is a significant correlation between response capacity at a low concentration of OA and serum OA-IgE antibody levels which cannot be observed when booster injections are performed with Perthrydal. Also note the very high degree of correlation observed between response capacity and OA-IgG_{2a} antibody levels in animals injected with Perthrydal at day 35.

Table 2 shows that in animals sensitized with 1 μg OA and two ampoules of Perthrydal and boosted with Al(OH)₃ day 35, there is a significant correlation between anaphylactic response capacity and OA-IgE serum levels day 48 which is not the case for corresponding saline-treated controls, despite the fact that the latter show higher response capacity (cf. Fig. 1B).

V. Effects of separate administration of adjuvant and antigen injections

Groups of animals were injected i.p. with 10 μg OA. Seven days later, injections of either 100 mg alum, two ampoules Perthrydal, 10 mg Silica gel, 10 μg OA, or saline were performed. Day 21 the animals were tested. Only those animals injected with alum or 10 μg OA at the second occasion showed a consistent response capacity, but the magnitude of this response was of low degree. Pretreatment of animals with alum or Perthrydal 7 days before injection of 10 μg OA (without Silica gel) did not lead to induction of a consistent response capacity as revealed by tests 14 days after OA-injections (results not shown).

DISCUSSION

In the present report we show that some agents, known to potentiate IgE-antibody and total IgE synthesis (alum and *B. pertussis* vaccine) when administered separately from antigen into primed but non-responding rats, induce the capacity in these rats to respond at antigen challenge *in vivo* with a bronchial anaphylactic reaction. Furthermore, under certain circumstances (when reactivity is precipitated by injections of alum) response capacity in such animals correlates to their serum OA-IgE antibody levels, but under other circumstances

(when reactivity is precipitated by injection of Perthydral) response capacity correlates to OA-IgG_{2a} antibody levels.

Several reports have previously shown that the nematode *Nippostrongylus brasiliensis*, *B. pertussis* vaccine, or HgCl₂, when suitably administered, potentiates ongoing IgE-antibody or total IgE synthesis in rats (e.g., 2, 5, 6, 10, 11, 12). These experiments have indicated that potentiation of IgE antibody synthesis by these agents might be limited to IgE-high responder strains (5, 11). Furthermore, in these strains, the nature of the adjuvant used at the priming injection of antigen determines whether a marked potentiation of the response can be achieved or not, and also whether a booster response can be achieved at a secondary antigen injection (5, 6). Thus, while primary IgE antibody responses to OA induced with *B. pertussis*, alum or Freund's complete adjuvant appear similar, potentiated responses following *N. brasiliensis* infection occur more consistently and at higher levels in animals primed with OA together with alum or Freund's complete adjuvant. On the other hand, significant IgE booster responses cannot be evoked in Hooded Lister rats if the adjuvant used in the priming event is alum or Freund's complete adjuvant, but only if *B. pertussis* is used (5, 6).

The results of the present report indicate that 1) a non-specific potentiation of the kind considered can also affect IgG_{2a} antibody synthesis, that 2) such a potentiation can also precipitate/induce increased response capacity in complete *in vivo* models of anaphylaxis and that 3) precipitation of response capacity can be accomplished in apparently anaphylactically naive rats which do not synthesize demonstrable amounts of IgE antibody. We realise that the rats not manifesting response capacity – those given 1 µg OA and Silica gel – have been primed with an antigen dose just below the threshold dose of OA able to induce a response. However, alum injections are performed 5 weeks after the priming injection of OA together with Silica, and these animals show no sign of reactivity either with respect to bronchial anaphylaxis or IgE antibody synthesis at either 14 or 35 days after immunization.

The mechanism of action behind the precipitation/potentiation of response capacity induced by alum or *B. pertussis* is not clarified by the present work. However, the characteristics of the effect suggest that both agents act at an immunological level. Conceivable mechanisms of action include a polyclonal activation of primed B cells, some of which are already committed to synthesis of OA-IgE antibodies, a functional elimination of some kind of mechanism (non-specifically acting suppressor cells) which otherwise effectively reduces synthesis of OA-IgE or OA-IgG_{2a} antibodies and total IgE, or a non specific enhancement of ϵ - and γ_{2a} isotype-specific T helper cells. However, elucidation of the precise mechanism of action of these adjuvants will require much further work. It is interesting to note that the broad interval between the priming injection of antigen together with Silica gel and the response capacity-precipitating injection of alum indicates that these two parts of the response, i.e., induction and expression, might be separately regulated. In these respects our data closely conform to those of Jarrett (5). Such an idea seems to imply a role for non-specifically acting suppressor cells. Finally, some other points of interest should be noted. 1) In animals primed with Silica gel adjuvant, Al(OH)₃ is much more effective in precipitation of OA-IgE-antibody and OA-IgG_{2a}-antibody (and *in vivo* bronchial anaphylactic) responses than Perthydral; the converse is true for potentiation of total IgE. 2) However, in animals primed with Perthydral adjuvant, alum injection leads to increased total IgE without affecting specific OA-IgE. The reason for this is not clear but indicates the existence of separate regulating systems for specific and total IgE biosynthesis. Similar observations have previously been reported concerning the effects of β -stimulating agents on IgE-antibody synthesis (9), the effect of HgCl₂ (11) and the potentiation of OA-IgE antibody (5).

Furthermore, in rats primed with 1 µg OA together with 10 mg Silica gel, median OA-IgE antibody levels do not significantly differ from zero until 13–14 days after the potentiation-inducing injection of alum. At that time bron-

chial anaphylactic response capacity (low antigen challenge dose) correlates to serum OA-IgE antibody levels. However, in rats primed with 10 µg OA together with 10 mg Silica, median OA-IgE-antibody levels increase already at day 38, i.e., 3 days after alum injection, and continue to rise until day 47–48, i.e., the test day. At this point there is no correlation at all between OA-IgE antibody level and bronchial reactivity. The reason for this is not clear, but the difference in kinetics of appearance of OA-IgE between the two groups suggests that "precipitation" and "potentiation" of OA-IgE antibody responses has to do with the different mechanisms of action of alum. These data also reinforce previous observations that bronchial anaphylactic response capacity in SD rats is closely correlated to serum OA-IgE antibody levels only under certain carefully chosen conditions (4).

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Bronchial Anaphylaxis in Actively Sensitized Sprague Dawley Rats: Studies on Mediators Involved**

By

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Abstract: Previous experiments have shown that SD rats actively sensitized to ovalbumin (OA) undergo bronchial anaphylaxis at intravenous challenge with a specific antigen. The inhibitory effect of antiallergic drugs in this experimental system and the relation of the response to serum reaginic antibody levels, depend on sensitization conditions (Dahlbäck 1981; Dahlbäck *et al.* 1982). The present experiments indicate that the main primary mediators of the respiratory anaphylaxis are serotonin and product(s) of the cyclooxygenase pathway of arachidonic acid metabolism. This is the case whether the animals are sensitized with 10 or 100 µg OA together with 100 mg alum and whether they are examined 2–3 or 5–6 weeks after sensitization. However, the bronchoconstrictory efficacy of exogenously added 5-HT varies with the immunization conditions of the animals: those given 100 µg OA together with alum are more reactive to 5-HT at intravenous challenge than animals given 10 µg OA and alum. Moreover, compounds known to affect the lipoxigenase pathway of arachidonic acid metabolism influence the bronchial anaphylactic reaction in different ways depending on immunization and test conditions. These data suggest that 5-HT and some not yet identified product(s) of cyclooxygenase/thromboxane synthetase are main mediators of allergen-induced bronchial anaphylaxis in the rat and that products of the lipoxigenase pathway play a minor direct role but may modulate the response.

Key-words: Bronchial anaphylaxis – mediators – rats.

Abbreviations used:

AA	Arachidonic acid
ACh	Acetylcholine
CPZ	Chlorpromazine
5-HT	5-Hydroxytryptamine (serotonin)
NDGA	Nordihydroguaiaretic acid
OA	Ovalbumin
PAF-acether	Platelet aggregating factor (L- α -lecithin, β -acetyl-O-alkyl)
SD	Sprague Dawley
SPF	Specific pathogen free
TxA ₂	Thromboxane A ₂

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Immunologically induced bronchial anaphylaxis in the rat is often used as an asthma model. In such studies actively or passively sensitized animals have been used. They are usually challenged by intravenous administration of antigen.

Active sensitization of the rats can be accomplished by injections of low doses of antigen together with alum and/or B. pertussis vaccine (Stotland & Share 1974a; Church & Miller 1978; Holme & Piechuta 1981; Brunet *et al.* 1983) or by infecting the animals with *Nippostrongylus brasiliensis* (Church *et al.* 1972; Church 1975a & b). These procedures are all known to lead to enhanced production of homocytotropic antibodies of the IgE-type (see Tada 1975; Jarrett 1978; Bazin & Pauwels 1982).

The mediators involved in these IgE-mediated anaphylactic reactions in the rat airway have been pinpointed. Church *et al.* (1972) Church (1975a), Stotland & Share (1974b), and Farmer *et al.* (1975) convincingly showed that methysergide, a 5-hydroxytryptamine (5-HT) antagonist, (partially) blocked the response. The effect of FPL 55712, a putative antagonist of slow reacting substance of anaphylaxis (SRS-A; i.e. leukotrienes LTC₄ and LTD₄), is less clearly documented (Farmer *et al.* 1975; Hamel *et al.* 1982; Piechuta & Holme 1982). Atropine was described as inhibitory (Stotland & Share 1974b, 0.1 mg/kg intravenously) or without effect (Church *et al.* 1972; 1 mg/kg intraperitoneally). Brunet *et al.* (1983) presented results from *in vitro* studies suggesting that the respiratory response of rats to antigen challenge is mediated by 5-HT in the trachea and by both 5-HT and leukotrienes in the lung parenchyma. These *in vitro* effects were also reflected *in vivo*. Cyclooxygenase inhibitors like aspirin and indomethacin have been attributed inhibitory capacity (Ahlstedt *et al.* 1983) implying a direct or indirect role also for cyclooxygenase products of arachidonic acid metabolism. On the other hand, antihistamines (e.g. mepyramine) and kallikrein inactivators (e.g. aprotinin) were by most authors considered almost ineffective. However, Stotland & Share (1974b) found that histamine contributed to the response in rats sensitized to OA together with both alum and B. pertussis vaccine.

With exogenously administered agonists,

Church (1975a) showed that 5-hydroxytryptamine, acetylcholine, and bradykinin were indeed potent bronchoconstrictors whereas SRS-A and prostaglandin F_{2α} were only slightly constrictory and histamine was without effect. The bronchoconstrictory effect of 5-HT could partially be reversed by atropine in a yet unsettled way. It may be noted that Aas (1983) recently presented results indicating that 5-HT stimulated isolated bronchial smooth muscle of the rat to contract via presynaptic receptors on cholinergic terminals in addition to postsynaptic receptors on the airway smooth muscles.

Vagal reflex pathways do not seem to play an important role in the development of the anaphylaxis (Stotland & Share 1974b; Farmer *et al.* 1975).

We previously observed that the inhibitory efficacy of a specified 'anti-allergic' agent was found to depend on the way of sensitization of the rats which were used for examination of the compounds (Dahlbäck 1981). Thus, disodiumcromoglycate and terbutaline were effective inhibitors in rats immunized with 1 or 10 µg OA and alum 6 weeks before test but they did not affect the response in animals challenged 2-3 weeks after immunization. In these systems, a significant correlation between the bronchial anaphylactic response and the antigen-specific IgE antibody levels was found only under a few of several carefully examined conditions. Bronchial anaphylactic responses did not correlate to serum antigen-specific IgG_{2a} antibody levels (Dahlbäck *et al.* 1982). These data indicated that reagin-mediated bronchial anaphylaxis in actively sensitized rats might be a heterogeneous phenomenon from an immunological point of view. This would then also provide an explanation of the inconsistency in the published data on the effects of atropine and dexamethasone (e.g. Church *et al.* 1972; Stotland & Share 1974b). To examine whether mediators might differ with immunization conditions, we immunized SD rats in different ways and examined in some detail the effect that various putative inhibitors of the action or production of possible mediators had on antigen-induced bronchial anaphylactic responses. The results of these studies form the basis of the present report.

Materials and Methods

Animals and immunization. Outbred male Sprague Dawley (SD) rats of SPF status were used. The animals were obtained from Møllegaards Avlslaboratorier, Ejby, Skensved, Denmark. At arrival the animals weighed about 200 g. They were kept at our laboratory under conventional animal housing conditions.

The animals were sensitized by an intraperitoneal injection of 10 or 100 µg of ovalbumin (OA; Sigma grade III, crystallized and lyophilized) in 0.5 ml saline mixed with 100 mg of dried and resuspended Al(OH)₃ (F 2200 Reheis Chemical Company; obtained through AB Astra, Södertälje). Some animals were examined unimmunized.

Respiratory measurements. At the indicated time after immunization the animals were used for test. They were anaesthetized with 40 mg/kg pentobarbital (Mebumal®, ACO, Sweden) and 25 mg/kg metomidate chloride (Hypnodil, LEO, Sweden) given intraperitoneally; 5 mg/kg succinylcholine (Celocurin®, Vitrum, Sweden) was given in order to prevent spontaneous respiration. Respiratory measurements were performed according to the method of Stotland & Share (1974a) slightly modified by Dahlbäck (1981). In brief, the trachea was cannulated and the rat ventilated with a Braun constant respiratory pump in a closed system. Intratracheal pressure was recorded through a side arm of the cannula connected to a Statham P23AC pressure transducer. The respiratory rate was set at 70 strokes/min. with a tracheal pressure of 8 cm H₂O (tidal volume range 2.0–3.75 ml). Throughout the entire experiment, mean arterial blood pressure was recorded by a Statham P23AC pressure transducer connected to a heparinized (Heparin®, Lövens, Sweden) catheter inserted in the right a. carotis. The blood pressure pulses were transmitted to a tachogram, where the heart rate was registered. All recordings were made on a Grass polygraph model 7B. The animals were challenged intravenously through a catheter inserted in the right jugular vein. A low antigen dose (0.3 mg OA to animals immunized with 10 µg OA and 0.5 mg OA to those immunized with 100 µg OA) was injected and the response recorded. Approximately 2 min. later an additional high dose (5 mg OA) was injected and the response again recorded. The intratracheal pressure increase resulting from each antigen injection was calculated as per cent of normal pressure. At the low antigen dose this increase was referred to as ΔP%, at the high antigen dose it was called ΔP max%.

Drugs were administered intravenously at the indicated times before antigen challenge. Some drugs were given to non-immunized animals for examination of direct effects. The sources of drugs were Sigma (acetylcholine (ACh), sodium salt of arachidonic acid (AA), bradykinin, chlorpromazine (CPZ), 5-hydroxytryptamine (5-HT), indomethacin, nordihydroguaiaretic acid (NDGA), 1-phenyl-3-pyrazolidone (phenidone)), Apoteksbolaget, Sweden (atropine sulphate, histamine dihydrochloride), Calbiochem (L-α-lecithin, β-acetyl-0-alkyl;

platelet aggregating factor (PAF-acether)) Recip, Sweden (prostaglandin F_{2α}), Sandoz Ltd., Switzerland (methysergide hydrogen maleate), Upjohn, U.S.A. (prostaglandins E₁ and E₂), EGA-Chemie, West-Germany (1-benzylimidazole), Wellcome Research, U.K. (BW 755 C; 3-amino-1-(3-trifluoromethyl)-phenyl-2-pyrazoline).

PAF-acether, AA, indomethacin, NDGA, and phenidone were dissolved and diluted in propylene glycol. Plain propylene glycol did not influence the response to antigen. Other drugs were dissolved in saline. Atropine, 1-benzylimidazole, BW 755C, chlorpromazine, methysergide, NDGA, and phenidone were all given 2 min. before antigen challenge; indomethacin was given 5 min. before challenge. For each drug, a similar amount of saline-treated or propylene glycol-treated control animals and drug treated experimental animals was tested at each occasion.

Mean values and standard error of the means are used for graphical illustration of the results. The inhibitory effects of the drugs were analyzed statistically by Student's t-test.

Results

Effects of exogenously administered putative mediators/bronchoconstrictors.

The dose-effect relation was examined for various putative mediators/bronchoconstrictors after intravenous administration to normal rats or rats immunized with 10 µg OA or 100 µg OA together with alum two to three or approximately six weeks before test.

Histamine at a dose of 10 or 100 µg/kg did not induce any demonstrable bronchoconstriction. Similarly, prostaglandins E₁ and E₂ at 10 µg/kg were not considered bronchoconstrictory. Prostaglandin F_{2α} had a slight bronchoconstrictory effect at 100–500 µg/kg (the recorded increase in intratracheal pressure was approximately 20% irrespective of immunization dose of OA or time after sensitization). Table 1 shows results recorded with acetylcholine, AA, bradykinin, 5-HT, and PAF-acether. Bronchoconstriction was induced by all these agents; maximum effective doses induced bronchoconstriction of a magnitude similar to that induced by maximum effective doses of antigen. Acetylcholine at 50 µg/kg was possibly slightly more effective as bronchoconstrictor in animals sensitized with 10 µg OA and alum than in normal animals or animals immunized with 100 µg OA and alum. On the other hand, at 100 µg/

Table 1.

Bronchoconstrictory effects of acetylcholine, arachidonic acid, bradykinin, 5-hydroxytryptamine, and PAF-acether. Constrictors were given intravenously at the indicated dose to normal SD rats or rats immunized with 10 µg OA or 100 µg OA two-three or six weeks before test. Figures represent mean $\pm$ S.E.M. (number of examinations).

Agent	Dose µg/kg	Increase in intratracheal pressure (%)				
		Normal	10 µg OA 2-3 weeks	100 µg OA + 100 mg Al(OH) ₃ 5-6 weeks	100 µg OA + 100 mg Al(OH) ₃ 2-3 weeks	100 µg OA + 100 mg Al(OH) ₃ 5-6 weeks
Acetylcholine	50	62 $\pm$ 6 (15)	77 $\pm$ 11 (11)	88 $\pm$ 9 (7)	51 $\pm$ 4 (30)	66 $\pm$ 5 (7)
	100	115 $\pm$ 8 (13)	113 $\pm$ 10 (10)	137 $\pm$ 9 (6) ^{NS}	76 $\pm$ 4 (49)***	79 $\pm$ 5 (20)***
AA	25000	110 $\pm$ 18 (12)	112 $\pm$ 9 (20)	113 $\pm$ 21 (10)	91 $\pm$ 10 (6)	103 $\pm$ 12 (15)
Bradykinin	400	65 $\pm$ 12 (6)	55 $\pm$ 9 (6)	61 $\pm$ 8 (4)	79 $\pm$ 18 (9)	66 $\pm$ 13 (6)
5-HT	50	108 $\pm$ 6 (22)	116 $\pm$ 6 (35)	137 $\pm$ 9 (11)**	139 $\pm$ 9 (13)**	156 $\pm$ 10 (14)***
PAF-acether	25	50 $\pm$ 8 (6)	51 $\pm$ 7 (6)	52 $\pm$ 5 (5)	74 $\pm$ 14 (6)	31 $\pm$ 6 (6)
	50	87 $\pm$ 6 (11)	93 $\pm$ 11 (6)	118 $\pm$ 10 (23)*	122 $\pm$ 11 (6)*	85 $\pm$ 16 (9)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

kg, acetylcholine was significantly less effective in the latter group of animals than in normal rats or rats given 10 µg OA and alum.

With bradykinin (at 400 µg/kg) and AA (at 25 mg/kg) there was no significant difference in the degree of bronchoconstriction between normal and sensitized animals. Similarly, the effect of 25 µg/kg of PAF-acether did not differ between normal and immunized animals. However, at 50 µg/kg PAF-acether induced a slightly higher degree of bronchoconstriction in rats given 10 µg OA and alum 6 weeks before test or 100 µg OA and alum 2-3 weeks before test than in normal animals. Finally, at 50 µg/kg, 5-HT induced a more severe bronchoconstriction in animals given 10 or 100 µg OA and alum 6 weeks before test and also in animals given 100 µg OA and alum 2-3 weeks before test than it did in normal rats.

Effect of repeated doses of bronchoconstrictors.

The respiratory parameters of animals given an injection of acetylcholine normalized within minutes. A second injection of an equal dose of acetylcholine 10 min. later most often induced a slightly higher increase in intratracheal response than the first dose; a third injection could be even more effective. Table 2 shows data from experiments with normal animals or animals sensitized with 10 or 100 µg OA together with 100 mg alum. At 50 µg/kg acetylcholine, immunized animals displayed a higher response than normal animals when examined 5-6 weeks after sensitization. At 100 µg/kg acetylcholine, the reduced reactivity recorded in animals immunized with 100 µg OA 2-3 weeks before test was blunted at the third acetylcholine injection. Thus, in general, sensitized animals examined 5-6 weeks after immunization showed increased response to repeated doses of acetylcholine compared to normal animals.

The bronchoconstriction induced by arachidonic acid was also more pronounced after a second and third dose than it was after the first one in normal animals. On the contrary, repeated injections of AA were generally less effective than the initial one in immunized animals (table 2). With PAF-acether (50 µg/kg), a bronchoconstrictory response could not be induced by a second injection of the agent after the return of the intra-

Table 2.

Effects on intratracheal pressure of repeated administration of acetylcholine and arachidonic acid. Rats immunized with 10 or 100 µg OA together with 100 mg alum two to three or four to six weeks before test were used. The response to one injection was allowed to return to the basal level before the next injection was given.

Drug and dose (µg/kg)	Immunization dose of OA (µg)	Test week		Increase in intratracheal pressure (%)		
				1st injection	2nd injection	3rd injection
Acetylcholine	50	Normal	n = 15	62 ± 6	77 ± 7	84 ± 7
	50	10 µg OA	n = 11	77 ± 11	80 ± 7	82 ± 6
	50	10 µg OA	n = 7	88 ± 9*	127 ± 9***	127 ± 11**
	50	100 µg OA	n = 7	66 ± 5	100 ± 12	108 ± 6*
	100	Normal	n = 13	115 ± 8	127 ± 7	133 ± 8
	100	10 µg OA	n = 7	115 ± 13	134 ± 15	125 ± 15
	100	10 µg OA	n = 6	137 ± 9	181 ± 9***	182 ± 15*
	100	100 µg OA	n = 49	76 ± 4***	104 ± 4**	123 ± 4
	100	100 µg OA	n = 20	79 ± 5***	113 ± 6	147 ± 7
Arachidonic acid	25000	Normal	n = 6	104 ± 22	162 ± 19	162 ± 17
	25000	10 µg OA	n = 5	89 ± 14	134 ± 35	91 ± 23*
	25000	10 µg OA	n = 5	71 ± 12	65 ± 9**	36 ± 8***
	25000	100 µg OA	n = 5	89 ± 12	83 ± 13**	37 ± 8***
	25000	100 µg OA	n = 10	76 ± 12	102 ± 15*	52 ± 10***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

tracheal pressure to baseline levels following the response to the first injection (not shown). With bradykinin (400 µg/kg) or with serotonin (50 or 100 µg/kg) the response to a second or a third injection of the mediator was not significantly different from the response to the first injection, neither in normal animals nor in any group of immunized animals (not shown).

Effects of putative blockers of action or function of endogenous mediators.

Acute drug effects on antigen-induced bronchial anaphylactic responses were examined in rats immunized with 10 or 100 µg OA two-three or six weeks before test. Challenge of the rats was performed after pretreatment with atropine, methysergide (a 5-HT blocker), 1-benzylimidazole (an inhibitor of thromboxane A₂ synthetase (Tai & Yuan 1978; Harris *et al.* 1980), 3-amino-1-(3-trifluoromethyl-phenyl)-2-pyrazoline hydrochloride (BW 755C; an inhibitor of both lipoxygenase and cyclooxygenase activity; Higgs *et al.* 1979; Humes *et al.* 1983), 1-phenyl-3-pyrazolidone (phenidone; an inhibitor of cyclooxygenase and lipoxygenase pathways in lung and platelets; Blackwell &

Flower 1978; see also Burka & Flower 1979), nordihydroguaiaretic acid (NDGA; an inhibitor of lipoxygenase activity which also affects cyclooxygenase; Hamberg 1976; Panganamala *et al.* 1977; Humes *et al.* 1983), indomethacin (an inhibitor of cyclooxygenase activity), or chlorpromazine. The used dose of each blocker was based on preliminary experiments in rats immunized with 100 µg OA and alum. A dose slightly lower than that giving an optimal effect was chosen. Antigen challenge was first performed with a low dose of OA (LD; 0.3 or 0.5 mg OA) and the effect was registered. Two min. later an additional dose of OA (HD; 5 mg OA) was given and the effect was again registered.

Fig. 1 gives the results for methysergide. At 50 µg/kg this agent was an effective inhibitor of bronchial anaphylactic reactions; there was no clear indication that its efficacy depends on the immunization or challenge conditions of the rats examined. Therefore, at each experimental condition, the effect of the drug has been calculated as per cent inhibition of the bronchial anaphylactic reaction recorded in vehicle-treated animals, and the four effect values thus obtained for each of

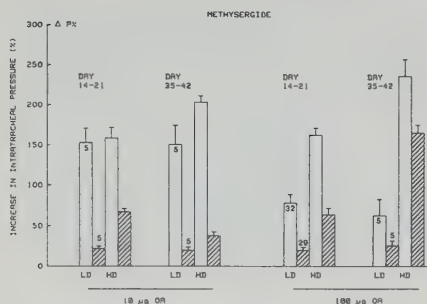


Fig. 1. Effect on bronchial anaphylactic responses of methysergide. Rats were immunized with 10 µg OA or 100 µg OA together with 100 mg Al(OH)₃ and were used for test 14-21 or 35-42 days later. Challenge was performed with a low dose of antigen (LD: 0.3 or 0.5 mg OA) and the effect was registered. Two min. later an additional dose of OA (HD: 5 mg OA) was given and the effect was again registered. Pretreatment was performed with saline (control animals, open columns) or methysergide (50 µg/kg, hatched columns). Figures given in columns indicate number of animals.

the two challenge doses of OA have been averaged. The results are given in fig. 2. Similarly, the effect of atropine (1 mg/kg) did not vary with

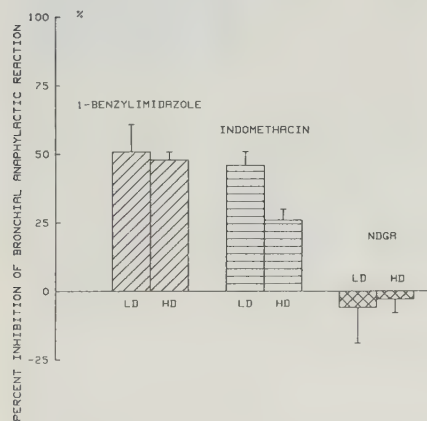


Fig. 3. Effect on bronchial anaphylactic responses of pretreatment with 1-benzylimidazole, indomethacin, or NDGA. Experimental conditions as specified in legend to fig. 2. Doses of drugs: 1-benzylimidazole: 10 mg/kg, Indomethacin: 5 mg/kg, NDGA: 10 mg/kg.

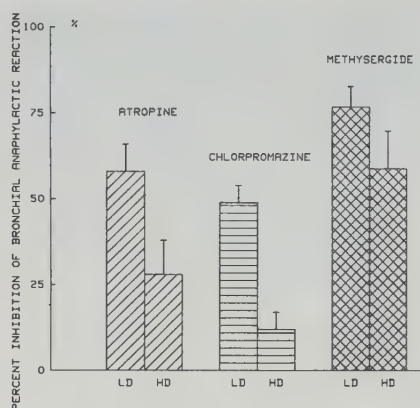


Fig. 2. Effect on bronchial anaphylactic responses of pretreatment with atropine (1 mg/kg), chlorpromazine (0.1 mg/kg) or methysergide (50 µg/kg). Rats were immunized with 10 µg OA or 100 µg OA together with 100 mg Al(OH)₃ and were used for test 14-21 or 35-42 days later. Challenge was performed with a low dose of antigen (LD: 0.3 or 0.5 mg OA) and the effect was registered. Two min. later an additional dose of OA (HD: 5 mg OA) was given and the effect was again registered. Figures given are mean values $\pm$ S.E.M. (n = 4) of per cent inhibition registered with drug-treated animals under each of four examined experimental conditions. Each of these four experiments consisted of at least 5 drug-treated and 5 vehicle-treated animals.

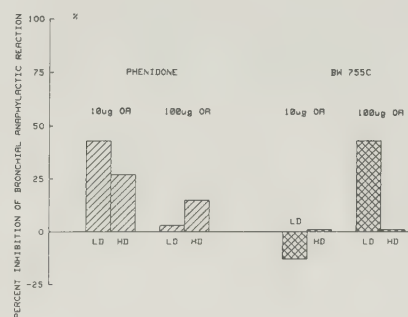


Fig. 4. Effects of bronchial anaphylactic responses of pretreatment with phenidone, or BW 755C. Experimental conditions as specified in legend to fig. 2. Doses of drugs: Phenidone 10 mg/kg, BW 755C 1 mg/kg. Figures given are mean values of 2 experiments each consisting of 5 drug-treated and 5 vehicle-treated animals.

experimental conditions. The averaged results for atropine (fig. 2) show that at the dose examined this drug partially blocked the anaphylactic bronchoconstriction. Chlorpromazine (0.1 mg/kg) was found to be only slightly less effective than methysergide and atropine as an inhibitor of bronchial anaphylactic reactions induced by 0.3 or 0.5 mg OA; however, the drug did only marginally affect the response induced by the high challenge dose of OA. The efficacy of chlorpromazine did not vary with immunization conditions.

The effects of putative inhibitors of the lipooxygenase – and/or cyclooxygenase pathways and of thromboxane-A₂-synthetase were also examined. Indomethacin and 1-benzylimidazole partially inhibited the bronchial anaphylactic reaction; again there was no indication that the efficacy of the drugs varied with immunization conditions. NDGA was found to be without effect at a dose of 10 mg/kg. The results are given in fig. 3. Note that for 1-benzylimidazole the inhibitory efficacy of the drug was equivalent at the two challenge doses of antigen which was not the case for atropine, methysergide or indomethacin and, as mentioned, certainly not for chlorpromazine.

The effects of phenidone and BW 755C were found to vary with immunization conditions of the animals. Thus, phenidone was more effective as an inhibitor in animals given 10 µg OA than in those sensitized with 100 µg OA apparently irrespective of the time chosen for test after immunization (fig. 4). With BW 755C the reverse seemed to be valid. With this compound there was a slight but nonsignificant enhancement of responses at the low provocation dose in animals given 10 µg OA. The results are given in fig. 4.

Effects of putative inhibitors of lipooxygenase/cyclooxygenase activities in combination with methysergide.

To estimate the effect of a combination of the 5-HT blocker methysergide and agents presumably affecting arachidonic acid metabolism, animals immunized with 10 µg OA and alum were given 5 µg methysergide and 2 min. later 10 mg/kg of phenidone or NDGA. As shown above (fig. 4), phenidone was a more effective inhibitor of bronchial anaphylactic reactions in this group of ani-

mals than in the group given 100 µg OA and alum. Fig. 5 gives the results. Both NDGA and phenidone increased slightly the inhibitory capacity of methysergide at a high challenge dose of OA but did not affect the response at the low antigen dose.

We also examined the effects of combinations of methysergide on one hand and 1-benzylimidazole, BW 755C, chlorpromazine, or indomethacin on the other. These experiments were performed with rats immunized with 100 µg OA and alum. The results are given in fig. 5.

The effects of indomethacin and 1-benzylimidazole were found to be additive to that of methysergide (compare fig. 3 to fig. 5). There was no indication that BW 755C affected inhibition induced by methysergide (compare fig. 5 and 2).

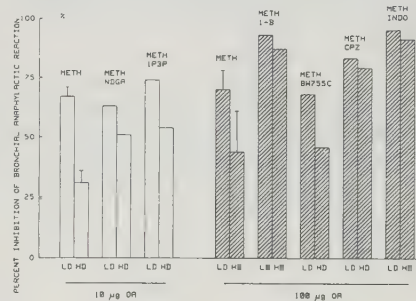


Fig. 5. Effects on bronchial anaphylactic responses of pretreatment of animals with combinations of methysergide and agents affecting AA metabolism. Doses of drugs: methysergide (5 µg/kg at 10 µg OA and 50 µg/kg at 100 µg OA), NDGA (10 mg/kg), phenidone (IP3P; 10 mg/kg), 1-benzylimidazole (1-B; 10 mg/kg), BW 755C (1 mg/kg), chlorpromazine (CPZ; 0.1 mg/kg), indomethacin (INDO; 5 mg/kg). Rats were immunized with 10 µg OA or 100 µg OA together with 100 mg alum as indicated at the bottom of the Figure and were used for test 3–5 weeks after immunization. Challenge was performed with a low dose of OA (LD: 0.3 or 0.5 mg OA) and the effect was registered. 2 min. later an additional dose of OA (HD: 5 mg OA) was given and the effect recorded. Indomethacin was given 5 min. before challenge. Methysergide and other drugs/vehicle were given 2 min. before antigen injections. Figures are mean values of per cent inhibition of 2 or more experiments each consisting of at least five drug-treated animals and 5 vehicle-treated control animals.

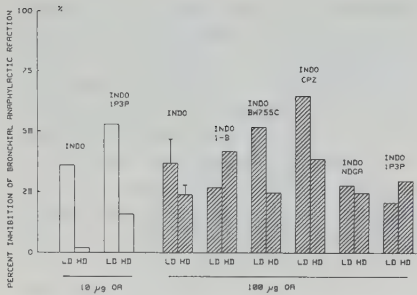


Fig. 6. Effects on bronchial anaphylactic responses of pretreatment of animals with combinations of indomethacin and other drugs affecting AA drug metabolism. Doses of drugs: Indomethacin (INDO; 5 mg/kg), 1-benzylimidazole (1-B; 10 mg/kg), BW 755C (1 mg/kg), chlorpromazine (CPZ; 0.1 mg/kg, NDGA (10 mg/kg); phenidone (IP3P; 10 mg/kg). Experimental conditions as given in legend to fig. 5.

Effects of putative inhibitors of lipoxygenase/cyclooxygenase or thromboxane A₂ synthetase in combination with indomethacin.

The effect of indomethacin alone was compared to the effect of combinations of this drug with 1-benzylimidazole, BW 755C, NDGA, or phenidone. Fig. 6 shows the results. In animals immunized with 10 µg OA, phenidone did not significantly affect the degree of inhibition recorded with indomethacin. In animals sensitized with 100 µg OA, the inhibitory effect of indomethacin at the high challenge dose of OA was enhanced by chlorpromazine (note that under these conditions chlorpromazine in itself is hardly inhibitory – fig. 2). The efficacy of indomethacin was not influenced by BW 755C, and was, if anything, reduced by 1-benzylimidazole, NDGA, and phenidone.

Effects of putative blockers of lipoxygenase and cyclooxygenase activity or antagonists of mediators on bronchoconstriction induced by arachidonic acid or PAF-acether.

Normal animals were pretreated with a vehicle or with 1-benzylimidazole, BW 755C, indomethacin, methysergide, NDGA, or phenidone and were challenged with arachidonic acid or PAF-acether

(fig. 7 gives the results). Despite a similar degree of bronchoconstriction obtained with these two constrictors (table 1) the inhibitory capacity of indomethacin, 1-benzylimidazole, phenidone, and BW 755C was slightly better when arachidonic acid was used as a bronchoconstrictor than if PAF-acether was employed. On the other hand,

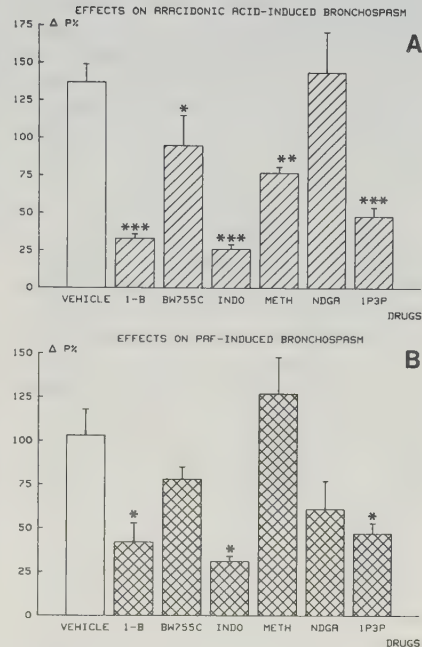


Fig. 7. Effects on bronchial anaphylaxis induced by A) arachidonic acid and B) PAF-acether of putative blockers of lipoxygenase- or cyclooxygenase activity or antagonists of mediators. Normal animals were pretreated with 1-benzylimidazole (1-B; 10 mg/kg), BW 755C (1 mg/kg), indomethacin (INDO; 5 mg/kg), methysergide (METH; 50 µg/kg), nordihydroguaiaretic acid (NDGA; 10 mg/kg), phenidone (IP3P; 10 mg/kg), or saline 2 min before challenge. Challenge was performed with 25 mg/kg intravenously arachidonic acid (frame A) or 50 µg/kg intravenously PAF-acether (frame B). Figures given are mean values of per cent inhibition recorded with at least five drug-treated animals and 5 saline-treated controls.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

NDGA affected (with borderline significance) only the bronchoconstriction induced by PAF-acether but not that induced by AA. Methysergide partially blocked the reaction induced by arachidonic acid but enhanced slightly that induced by PAF-acether.

Effect of vagotomy.

In one group of animals, immunized with 100 µg OA and alum 3 weeks before test, bilateral vagotomy was performed 10 min. prior to challenge with OA. The response of this group of animals to OA was compared to that of sham operated rats similarly challenged. Vagotomy did not affect the response to a high challenge dose of OA.

Discussion

It is known that 5-HT is a main mediator of bronchial anaphylactic reactions in actively sensitized rats, that products of arachidonic acid may contribute to the response, whereas histamine is of minor importance (for ref. see Introduction).

As previous studies have shown, rats differ in response to anti-allergic drugs according to immunization conditions. The aim of the present work was to examine whether various mediators and arachidonic acid products contribute in different ways to a bronchial anaphylactic response induced in rats sensitized according to these different regimens.

In our experiments, the rats were deeply anaesthetized. The failure of bilateral vagotomy to alleviate antigen-induced bronchoconstriction supports the opinion (Farmer *et al.* 1975; Stotland & Share 1974b) that the response in such anaesthetized animals is not under vagal control. This does not exclude a role for vagal influences in conscious or lightly anaesthetized spontaneously breathing animals.

First, the effects of exogenously administered putative mediators or mediator precursors were examined in normal and immunized rats. The results (table 1) agree well with previously published results (e.g. Church 1975a). Thus, 5-HT and acetylcholine but not histamine were found to be efficient bronchoconstrictors. Pauwels *et al.* (1983)

showed that inbred rat strains differ in bronchial reactivity to 5-HT independently of their IgE-producing capacity. Church (1975a) and Brunet *et al.* (1983) failed to find any *significant* difference in efficacy of acetylcholine and 5-HT, respectively, between normal and immunized animals. We noticed a slight but non-significant increase in response to 50 µg/kg acetylcholine in rats given 10 µg OA and alum. A similar observation was recorded by Church (1975a) with rats sensitized to *Nippostrongylus brasiliensis* five weeks before test. More unexpectedly, we found that animals given 100 µg OA and alum but not those given 10 µg OA and alum showed reduced responsiveness to 100 µg/kg acetylcholine but increased responsiveness to 5-HT. The first mentioned observation seems to contrast with the report of hyper-reactivity to cholinergic stimuli observed in IgE-producing rabbits (Halonen *et al.* 1983) or allergic humans (Smith *et al.* 1980); the last-mentioned observation is in contrast to the reduced responses to 5-HT recorded with BN × Wi/Fu rats immunized by exposure to aerolized OA (Ahlstedt *et al.* 1983). The reasons for these apparent discrepancies are not clear but experimental conditions might contribute. Thus, most of our immunized animals were found to express an unusual kind of increasing responsiveness to acetylcholine with repeated challenges (table 2). The variation of this effect in various groups of rats suggests that the response to acetylcholine is in fact modulated by immunization conditions of the animals. Only further experiments can reveal the actual mechanism behind this effect.

Arachidonic acid and PAF-acether were also found to induce dose-dependent bronchoconstriction. The effect of a single injection of AA did not differ in normal or immunized animals. On the other hand, PAF-acether at 50 µg/kg tended to induce a slightly higher response in some groups of immunized animals than it did in normals (table 1). Contrary to the effect seen with acetylcholine, challenge of sensitized but not normal animals with AA induced partial desensitization to a repeated dose. The reason for this is not clear. One may speculate that physiologically active products of arachidonic acid (leukotrienes, thromboxanes etc.) might be more effectively degraded

in immunized animals due to increased activity of inflammatory cells (e.g. by hydroxyl radical inactivation, Henderson & Klebanoff 1983).

PAF-acether induced complete unresponsiveness to a further dose. Desensitization has previously been recorded by others (Camussi *et al.* 1983), its mechanism is not yet revealed.

Then we examined putative mediator blockers, cyclooxygenase inhibitors, compounds reported to inhibit cyclooxygenase and lipoxygenase activities and a putative inhibitor of thromboxane A₂ production. We also examined the effect of chlorpromazine, an agent with various reported effect mechanisms, e.g. α -receptor blocking activity (for review see Patil & Ruffolo 1980), inhibition of protein kinase C activity (Mori *et al.* 1980), or phospholipase C activity (Wightman *et al.* 1981) or calmodulin binding (Levin & Weiss 1979). Immunization conditions did evidently not influence the effect of the two mediator blockers. Thus, methysergide and, as previously shown by Stotland & Share (1974b), atropine were effective inhibitors of the anaphylactic reaction. The physiological basis for the effect of atropine is not clear. It may block contraction induced by 5-HT stimulation of presynaptic receptors on cholinergic terminals (cf. Church 1975a; Aas 1983) or it may act directly on the smooth muscle, blocking the action of allergen-induced release of acetylcholine.

Indomethacin and 1-benzylimidazole inhibition does not vary with the form of sensitization. The effect we see with indomethacin is similar to that described by Ahlstedt *et al.* (1983) in animals sensitized by repeated exposure to aerosolized OA. In contrast, Stotland & Share (1974b) and Farmer *et al.* (1975) failed to find any inhibition of rat lung anaphylaxis in actively or passively sensitized animals with indomethacin (1 mg/kg) and aspirin (5 mg/kg), respectively. In the guinea-pig, indomethacin (5 mg/kg) slightly enhances antigen-induced bronchoconstriction *in vivo* (Andersson 1982) and antigen-induced tracheal contraction *in vitro* (Burka 1983). 1-Benzylimidazole is relatively more effective at the high provocation dose than indomethacin suggesting that thromboxanes might contribute to the response elicited at high antigen doses of provocation.

On the contrary, the effects of some but not all

compounds presumably affecting AA metabolism seem to depend on immunization conditions of the animals as detailed in figs. 3–6. The lipoxygenase inhibitors examined are equipotent as inhibitors of the enzyme of rat basophilic leukaemia cells (Levinson & Heidi 1983). However, in the present system they show different efficacy. BW 755C is active only in animals immunized with 100 μ g OA, phenidone is active primarily in animals immunized with 10 μ g OA, whereas NDGA fails to display inhibitory activity. The reason for these differences in inhibiting capacity cannot readily be explained at present. It may be noted that in guinea-pig parenchymal tissue *in vitro* NDGA and phenidone inhibit antigen-induced contractions (Burka 1983). Antigen-induced bronchial anaphylactic reactions in guinea-pigs *in vivo* are at most slightly affected by BW 755C, NDGA, or phenidone (Andersson 1982; Lewis *et al.* 1983).

Combinations of methysergide with indomethacin or 1-benzylimidazole block completely the anaphylactic reaction at both provocation doses (fig. 5). On the other hand, combinations of methysergide with phenidone, NDGA, or BW 755C are at most, only slightly more inhibitory than methysergide alone. Piechuta & Holme (1982) reported that NDGA and BW 755C, alone or in combination with methysergide, produced a dose dependent inhibition of OA-induced dyspnoea. In their experiments, BW 755C showed an ED₄₀ value of 4.8 (mg/kg intravenously) in tests in combination with methysergide. In our system 1 mg/kg intravenously of BW 755C is without effect when effects of combinations of indomethacin and the other examined drugs were compared with the effect of indomethacin alone (fig. 6), only a combination of chlorpromazine and indomethacin increased the degree of inhibition compared to that recorded with either drug alone.

Finally, bronchoconstrictory effects induced by AA or PAF-acether were partially characterized with respect to the effect of inhibitors (fig. 7). The response to both agents could be reduced by reasonable doses of a number of different compounds suggesting that the effects of both AA and PAF-acether were at least in part indirect. Thus, the effect of both drugs was to a major part reduced by indomethacin.

It is noteworthy that AA-induced bronchoconstrictions are partially blocked by methysergide but not affected by NDGA – in this respect AA-induced bronchoconstrictions resemble antigen-induced ones. On the other hand, PAF-acether-induced bronchoconstrictions are partially blocked by NDGA but, if anything, enhanced by methysergide. This observation would suggest that 1) the effect of exogenously administered PAF-acether is direct and not involving mast cell mediator (5-HT) release, 2) that endogenously released PAF-acether could play a minor role as mediator of antigen-induced bronchial anaphylactic reactions under the present circumstances, and 3) that the absence of effect with NDGA on antigen-induced bronchoconstrictions is not due to insufficient dosing of the drug since it shows efficacy against PAF-acether.

In conclusion, we find the most likely explanation of these data to be that 'primary' mediators of the anaphylactic bronchoconstriction in the rat (the main mediators being 5-HT, possibly acetylcholine, and some cyclooxygenase derived products of arachidonic acid) do not differ in effect with immunization condition. However, the production of or the release from mast cells (?) of these mediators might be influenced by products of arachidonic acid, the synthesis/release of which might be influenced differentially by NDGA, BW 755C or phenidone depending on immunization and provocation conditions of experimental animals.

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V

V

ANTIGEN-INDUCED BRONCHIAL ANAPHYLAXIS IN
ACTIVELY SENSITIZED SD RATS:
EFFECTS OF GLUCOCORTICOID TREATMENT

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- glucocorticosteroids - rats

Abbreviations used:

BAR	Bronchial anaphylactic reaction
BPB	p-Bromophenacyl bromide
BUD	Budesonide
DEX	Dexamethasone
GCS	Glucocorticosteroid(s)
HC	Hydrocortisone
OA	Ovalbumin
PMN	Polymorphonuclear
QDC	Quinacrine dehydrochloride
SD	Sprague Dawley
SPF	Specific pathogen-free

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SUMMARY

We examined the effects of glucocorticosteroids (GCS) on antigen-induced bronchial anaphylactic reactions (BAR) in SD rats immunized with ovalbumin (OA) and alum. The animals were treated with vehicle, budesonide (BUD), dexamethasone (DEX), or hydrocortisone (HC) at various times before intravenous (i.v.) antigen challenge. The drugs were administered either intraperitoneally (i.p.) or intratracheally (i.t.); the BAR was elicited by a low or by a high challenge dose of antigen.

A BAR elicited by a low challenge dose of antigen was reduced in a dose-dependent way by all GCS after i.p. administration; at 1 mg/kg, BUD and DEX significantly reduced BAR and at 50 mg/kg all three of the examined compounds inhibited the BAR by 50 per cent or more. For BUD maximum effect was recorded when it was given 12 hours before test. There was only a slight variation in inhibitory effects of the GCS with immunization conditions of test animals. I.t. instillation of the drugs did not markedly increase their inhibitory capacity as compared to i.p. administration.

BAR elicited by a high antigen dose was at best marginally affected by the GCS either when given i.p. or i.t.. Thus, antigen-induced airway reactivity in rats can be reduced by GCS-treatment provided that this is performed a sufficient time before test and that the challenge dose of antigen is not too high.

Clinical aspects

Bronchial anaphylactic reactions induced by a low antigen dose in actively sensitized rats are reduced if the animals are pretreated with a single dose of a potent GCS 5 hours or more before challenge. These results may contribute to the understanding of the recently reported 'anti-allergic' effect of GCS in asthmatics.

INTRODUCTION

The development of optimal anti-asthma drugs requires reliable animal models of the disease. For screening purposes, asthma models in small laboratory animals are desirable. However, to date no such model exists which can clearly account for both the immunological aspects of human allergic asthma and the nonspecific airway hyperreactivity which is a characteristic of various forms of asthma.

Bronchial anaphylaxis in sensitized rats is one asthma model which has been used by many authors. In general, it does not encompass the nonspecific hyperreactivity-component of the human disease. However, the antigen-induced bronchial contraction in rats is presumably mediated by the IgE-type of reaginic antibodies fixed to mast cells in the airways. Thus, this model probably shares some of the immunological characteristics of the immediate reaction in human asthma. Several variants of the rat bronchial anaphylaxis model exist.

The reported effects of drugs in these rat models of asthma are not always consistent. This applies for example to effects of the glucocorticosteroid (GCS) dexamethasone (6,38). In part, this may be due to differences in timing of administration of the drug. However, other factors, such as antigen dose at challenge, may also contribute. Moreover, we have previously shown that the inhibitory capacity of specified 'anti-allergic' asthma drugs may in fact vary with immunization conditions (11). For example, disodium cromoglycate (DSCG) is an effective inhibitor of anaphylaxis in rats immunized with 1 or 10 ug OA and alum 6 weeks before challenge but it is less effective in reducing the anaphylaxis in similarly sensitized animals challenged with the low dose of antigen 2-3 weeks after immunization. Moreover, it does not reduce the airway reactions elicited by high allergen doses.

Inhaled GCS, such as budesonide, can reduce the allergen-induced immediate bronchoconstriction in asthmatics and in allergic sheep (14). However, information on this effect of GCS is conflicting (30). It may require longer pretreatment times than the better documented attenuation of late reaction (10,16,28). Whether the

inhibitory effect of GCS on the immediate bronchoconstriction is due to 'anti-allergic' (i.e., mediator release inhibiting) properties of the compounds is not clarified. Indeed, GCS treatment reduces the peak plasma level of histamine and the per cent increase in serum neutrophil chemotactic activity at allergen challenge of grass or ragweed pollen sensitive asthmatics (26). However, in vivo treatment with GCS does not seem to reduce responsiveness of basophils to immunological stimuli when assessed in vitro; rather, GCS treatment reduces blood levels of basophils (31). On the other hand, dexamethasone can reduce immunologically induced basophil (2,32,33) or mouse peritoneal mast cell (9) - but not human lung tissue (34) - histamine release provided that the cells before challenge are incubated with the steroid overnight in vitro. In the rat mast cell system even ionophore (A23187)-induced release of mediators is reduced by in vivo treatment with DEX (25). Moreover, in rats and guinea pigs treated in vivo with GCS one day or more before test, inhibition of antigen-induced bronchial anaphylactic reactions (1,6,38) and antigen-induced release of various anaphylactic mediators from chopped lung tissue or serosal mast cells has been reported (25,35).

We therefore examined in some detail conditions under which GCS treatment could affect bronchial anaphylactic reactions in the rat model of asthma which we have previously characterized.

We also asked whether the inhibitory capacity of a GCS could vary with sensitization conditions of the animals. Furthermore, since the characteristics of the inhibitory actions of budesonide, dexamethasone, and hydrocortisone were found to differ slightly in the human basophil histamine release system (2) these compounds were chosen for tests.

MATERIALS AND METHODS

Animals and immunization. Outbred male Sprague Dawley (SD) rats of SPF status (Møllegaards Breeding Center Ltd, Ejby, Skensved, Denmark) were used at a weight of 200 g. After arrival at our laboratory the animals were kept under conventional animal housing conditions. They were sensitized by an i.p. injection of 10 µg or

100 μ g ovalbumin (OA; Sigma grade III, crystallized and lyophilized) in 0.5 ml saline mixed with 100 mg of dried and resuspended $\text{Al}(\text{OH})_3$ (F 2200 Reheis Chemical Company; obtained through AB Astra, Södertälje).

Respiratory measurements. At the indicated time after immunization, the animals were used for test. They were anaesthetized with 40 mg/kg pentobarbital (Mebumal^R, ACO, Sweden) and 25 mg/kg metomidate chloride (Hypnodil^R, LEO, Sweden) given i.p.. 5 mg/kg succinylcholine (Celocurin^R, Vitrum, Sweden) was given i.p. in order to prevent spontaneous respiration. Respiratory measurements were performed according to the method of Stotland & Share (37) slightly modified by Dahlbäck (11). In brief, the trachea was cannulated and the rat ventilated with a Braun constant respiratory pump in a closed system. Intratracheal pressure was recorded through a side arm of the cannula connected to a Statham P23AC pressure transducer. The respiratory rate was set at 70 strokes/min with a tracheal pressure of 8 cm H_2O (tidal volume range 2.0-3.75 ml). All recordings were made on a Grass polygraph model 7B. The animals were challenged through a catheter inserted in the right jugular vein.

Challenge was performed by injection of a low antigen dose (LD; 0.3 mg OA to animals immunized with 10 μ g OA and 0.5 mg OA to those immunized with 100 μ g OA) and the response recorded. Approximately 2 min later (when the response to the low antigen dose had levelled) an additional high dose (HD; 5 mg OA) was injected and the response again recorded. The intratracheal pressure increase resulting from each antigen injection was calculated as per cent of prechallenge pressure. At the low antigen dose this increase was referred to as $\Delta P\%$, at the high antigen dose it was called $\Delta P_{\text{max}}\%$.

Drugs. Drugs were administered i.v., i.p. or by i.t. instillation at the indicated time before antigen challenge. The sources of drugs were Sigma (dexamethasone, DEX; quinacrine dihydrochloride, QDC), Apoteksbolaget, Sweden (hydrocortisone, HC), AB Draco, Sweden (budesonide, BUD; p-bromophenacyl bromide, BPB), Fisons Ltd., UK (disodium cromoglycate, DSCG, Lomudal^R).

For i.p. or i.v. administration drugs were dissolved and diluted in propylene glycol, except DSCG and QDC which were dissolved in 0.9% NaCl. Propylene glycol in itself did not influence the response to antigen. Drugs to be administered by i.t. instillation were suspended in a vehicle composed of Tween 80 0.04%, NaCl 0.65% and carboxymethyl cellulose 0.75% in water. Fresh suspensions were prepared once a week, the suspensions being stored at +5°C. The volume administered i.t. was 0.05 ml/100 g body weight. The vehicle in itself did not influence the response to antigen. DSCG, BPB and QDC were given i.v. at the indicated time before challenge.

In each experiment a similar number of vehicle-treated control animals and drug-treated experimental animals were tested from the same sensitization pool. Mean values of $\Delta P\%$ and $\Delta P_{max}\%$ were calculated for each group. Per cent inhibition of the anaphylactic reaction was calculated as follows:

$$\% \text{ inhibition of BAR} = 100 \times \left(1 - \frac{\text{Mean } \Delta P\% \text{ or } \Delta P_{max}\% \text{ of drug-treated animals}}{\text{Mean } \Delta P\% \text{ or } \Delta P_{max}\% \text{ of vehicle-treated animals}} \right)$$

Figures given show mean values $\pm$ S.E. for % inhibition of BAR calculated from three - seven experiments each of which included at least three drug-treated and three vehicle-treated animals.

Serum OA-IgE, OA-IgG_{2a} antibody, and total IgE levels were determined as described (17).

RESULTS

Variation in anti-anaphylactic effect of GCS with immunization conditions?

We initially asked whether glucocorticosteroids could affect antigen-induced bronchial anaphylaxis in actively sensitized SD rats and, if so, whether effects of the GCS varied with immunization conditions. We therefore chose to examine a comparably high dose of the GCS, 10 mg/kg, given i.p. one day before test. Experiments

were performed with animals immunized with 10 µg or 100 µg OA together with 100 mg of alum 2-3 or 5-6 weeks before test. This dose and time of administration of drugs and the sensitization conditions were chosen on the basis of pilot experiments and previously recorded results (6,8,11). Figure 1 gives the results.

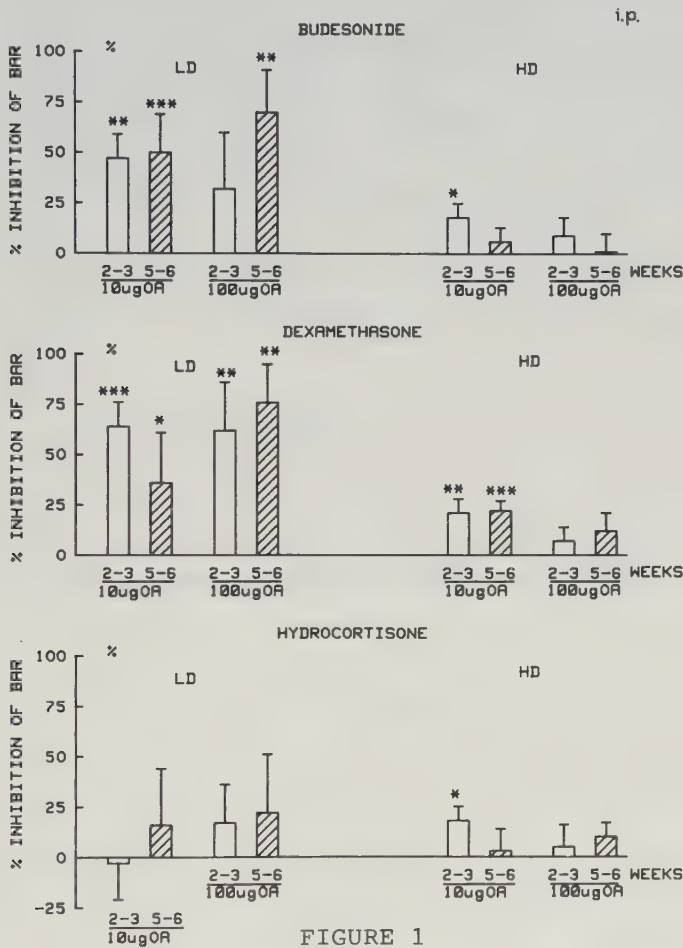


FIGURE 1

Inhibition of BAR by i.p. treatment with GCS

Rats were immunized with 10 µg or 100 µg OA and 100 mg alum and were used for test 2-3 weeks or 5-6 weeks later. Challenge was performed as described in Material and Methods. Pretreatment was performed i.p. with vehicle or 10 mg/kg of the indicated GCS the day before test. Effects of GCS are shown as per cent inhibition of response recorded in vehicle-treated animals + S.E. (see Materials and Methods). Significance of inhibition was assessed by analysis of variance with linear contrasts on vehicle- and drug-treated animals.

* P < 0.05 ** P < 0.01 *** P < 0.001.

The following findings merit comments: firstly, in general BUD and DEX are effective inhibitors of a BAR elicited by a low antigen dose whereas the inhibitory effect of HC at this dose is marginal. A BAR elicited by a high antigen dose is much less reduced by the GCS treatment. Secondly, at the low antigen challenge dose, BUD and DEX seem to be equally effective inhibitors irrespective of the immunization dose of antigen employed and the time of test. Thirdly, at a high challenge dose of antigen, BAR in animals immunized with 100 ug OA are not significantly affected by GCS. However, BAR in animals immunized with 10 ug OA 2-3 weeks before test are significantly albeit slightly reduced by all three examined GCS. The efficacy of HC under these conditions relative to that of BUD and DEX contrasts with the lack of activity of HC when BAR is elicited by a low antigen dose.

Dose-relation for effect of i.p. administered GCS

The dose-relation for the inhibitory effect of i.p. administered GCS was examined in animals immunized with 10 ug OA and alum 5-6 weeks before test. These conditions were chosen since DEX also reduced the high dose antigen-induced BAR in such animals. The results are shown in Figure 2. Again, BUD and DEX tend to show equal efficacy. Note that at 50 mg/kg, HC is nearly as effective as the other two GCS in reducing a BAR elicited by a low antigen dose. Even at 50 mg/kg none of the GCS reduced a BAR elicited by a high antigen dose more than marginally.

Effect of 'long-term' treatment with a low dose of BUD on BAR

With reference to effects of repeated administration of GCS in asthma (5,29,36), we were interested to know whether BUD could affect the BAR after 'long-term' administration followed by a short wash out period. Therefore, animals immunized with 10 ug OA together with alum were given 0.01 mg/kg or 0.1 mg/kg BUD, or vehicle i.p. five days a week for three weeks from day 26 to day 44 after sensitization and were challenged one week later, day 51. Neither BAR induced by a low nor that induced by a high antigen dose differed between BUD- and vehicle-treated animals in any respect. Moreover, significant alteration in specific OA-IgE, OA-IgG_{2a}, serum antibody or total IgE levels were not seen in drug-treated groups compared with controls (results not shown).

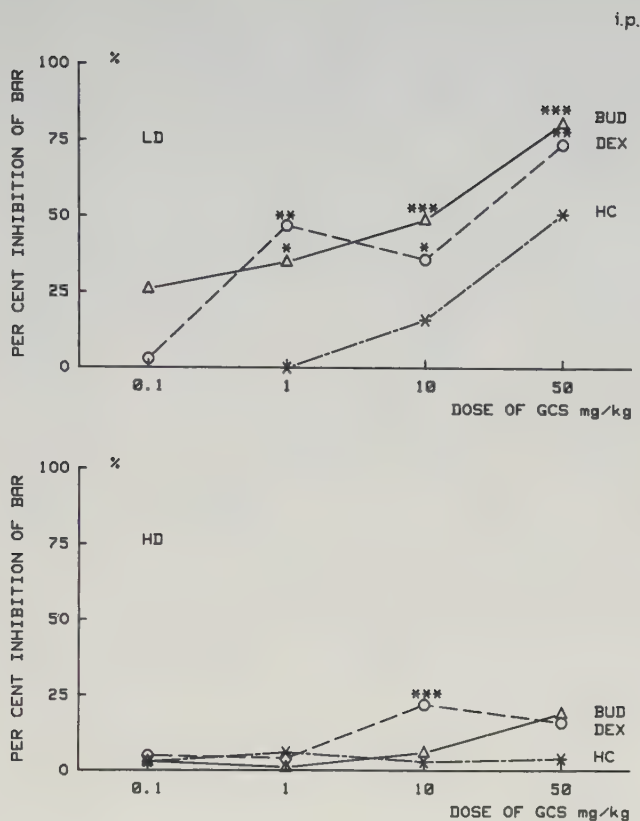


FIGURE 2

Dose-response relationship for inhibitory effects of i.p. administered GCS on antigen-induced reactions.

Rats were immunized with 10 μ g OA 5-6 weeks before test. Challenge was performed as described in Material and Methods. Treatment was performed with vehicle or the indicated dose of GCS the day before test. Effects are shown as per cent inhibition of BAR of vehicle-treated animals (see Material and Methods). Statistics as described in legend to Figure 1.

Influence of time of i.p. administration on effect of GCS

We examined with BUD (10 mg/kg) the influence of the time of its administration on the inhibitory effect of a GCS on the BAR. Experiments were performed with animals given 10 μ g OA either 2-3 or 5-6 weeks before test. Since the results did not differ for these two groups of animals, the combined results are given (Figure 3). At a low challenge dose of OA, BUD inhibited BAR

significantly when given 5, 12, or 18-24 h before test. Unexpectedly, at a high allergen challenge dose, a 5 h pretreatment period with BUD resulted in a significant increase in the BAR.

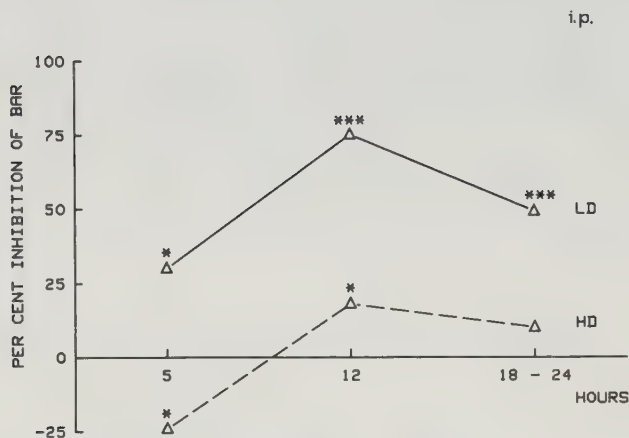


FIGURE 3

Influence of time of i.p. administration of BUD on its effect on BAR.

Rats were immunized with 10 μ g OA and alum and were used for tests 2-3 or 5-6 weeks later. Challenge was performed as described in Material and Methods. Pretreatment was performed i.p. with 10 mg/kg of BUD at the indicated time before test. Effects are given as per cent inhibition of vehicle-treated animals (see Material and Methods). Statistics as in Figure 1.

Influence of dose and time of i.t. administration on effect of GCS on BAR

We then examined whether locally administered GCS could reduce a BAR. Animals immunized with 10 μ g OA and alum 2-3 weeks before test were given GCS by i.t. instillation one day before test. As shown above, under these immunization conditions all examined GCS at 10 mg/kg i.p. significantly reduced the BAR elicited by a high challenge dose of antigen (Fig. 1). Figure 4 shows the

results. Again, BUD and DEX tended to show similar efficacy in reducing a BAR induced by a low antigen dose. Optimum effects were reached at 2.5 mg/kg. BUD and DEX could also reduce a BAR elicited by a high antigen dose; possibly the latter drug is now

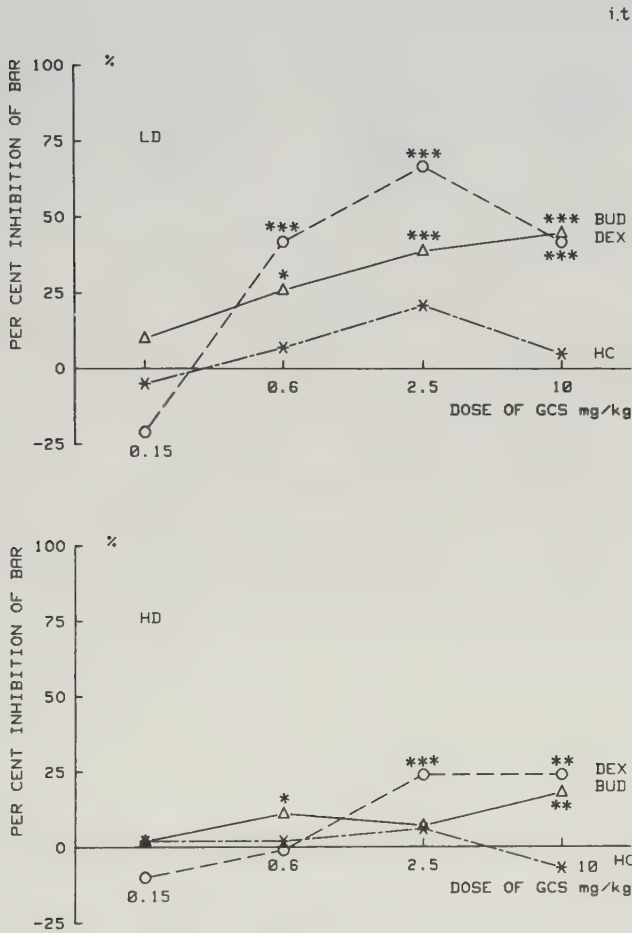


FIGURE 4

Dose-response relationship for inhibitory effects of i.t. administered GCS on antigen-induced reactions.

Rats were immunized with 10 µg OA and alum 2 weeks before test. Challenge was performed as described in Material and Methods. Pretreatment was performed by i.t. administration of vehicle or the indicated dose of GCS the day before test. Effects of GCS are shown as per cent inhibition of response recorded in vehicle-treated animals. Each figure is based on at least two experiments, each consisting of at least four drug-treated and four vehicle-treated animals. Statistics as in legend to Figure 1.

somewhat more effective than the former. HC i.t. did not significantly reduce the BAR elicited either by a low or by a high dose of antigen. Figure 5 shows effects of 2.5 mg/kg of each GCS as a function of the time of its administration. Note that HC did effectively reduce a BAR elicited by a low antigen dose, if given 1 or 5 h before test.

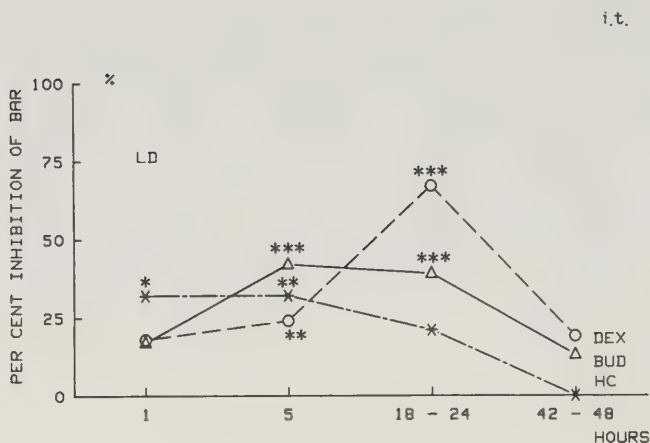


FIGURE 5

Influence of time of i.t. administration of GCS on its effect on BAR.

Rats were sensitized with 10 µg OA and alum and were used for tests 2-3 weeks later. Challenge was performed as described in Material and Methods. Pretreatment was performed with 2.5 mg/kg of each GCS at the indicated time before test. Effects of GCS are given as per cent inhibition of response recorded in vehicle-treated animals. Each figure is based on at least two experiments each consisting of at least five drug-treated animals and at least five vehicle-treated animals. Statistics as in legend to Figure 1.

Effect on the BAR of a combination of BUD and DSCG

A previous report (38) described a synergistic inhibitory effect of DSCG and DEX on the BAR. We presently examined the effect of a combination of DSCG and BUD in relation to those of each drug

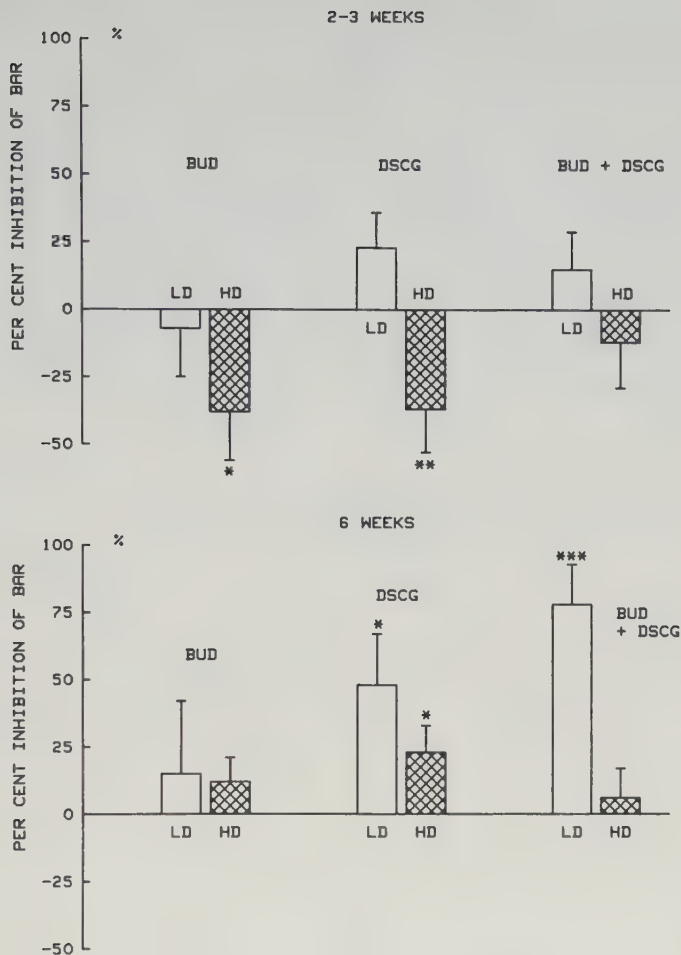


FIGURE 6

Inhibition of BAR by BUD and DSCG alone or in combination.

Rats were immunized with 10 μ g OA and alum and were used for test 2-3 or 6 weeks after sensitization. Challenge was performed as described in Material and Methods. Treatment was performed with vehicle or BUD (1 mg/kg i.p.), with DSCG (1 mg/kg i.v.), or with BUD and DSCG. BUD was given 18-24 h and DSCG 1 min before challenge. Effects are shown as percent inhibition of BAR of vehicle-treated animals. Each figure is based on 3 experiments, each consisting of at least 3 drug-treated and a similar number of vehicle-treated animals. Statistics as described in legend to Figure 1.

separately. Animals were sensitized with 10 μ g OA and alum and were examined 2-3 or 6 weeks after sensitization. Our previous experience is that DSCG is an effective inhibitor of BAR elicited by a low challenge dose of antigen in such animals at the latter but not at the former time point (11). The present results (Figure 6) show that BUD at the dose 1 mg/kg i.p., in itself marginally effective, augments the anti-anaphylactic efficacy of DSCG at a low (but not at a high) antigen challenge dose in rats examined 6 weeks (but not in those examined 2-3 weeks) after sensitization. Unexpectedly, both BUD and DSCG, but not the combination of those drugs, enhanced the BAR in rats challenged with a low antigen dose 2-3 weeks after sensitization.

Effects of putative phospholipase A_2 -inhibitors

The mode of the anti-inflammatory action of GCS has not been fully clarified. One hypothesis is that GCS can induce the synthesis/release of a protein or proteins (macroscortin/lipomodulin) which then mediate(s) the effect by inhibiting a phospholipase A_2 activity. This would lead to inhibition of the triggering by various stimuli of release of arachidonic acid from phospholipids (for ref. see 4,19). Therefore we examined the effects on BAR of BPB and QDC, two compounds which also are reported to inhibit phospholipase A_2 activity. Figure 7 shows the results. BPB at 10 mg/kg exerted no inhibitory effect but in animals given 10 μ g OA, BPB tended to enhance the response. At this dose, the drug itself increased the BAR in many animals. At 0.1 mg/kg or 1 mg/kg of BPB no effect was recorded (not shown). QDC at 1 mg/kg possibly affected the BAR in animals given 10 μ g OA at immunization; a higher dose of QDC (10 mg/kg) if anything decreases the inhibitory efficacy (not shown). BAR elicited by a high antigen challenge dose were also partially affected by QDC.

DISCUSSION

The present work shows that GCS can inhibit allergen-induced bronchial anaphylactic reactions in actively sensitized SD rats provided that the drugs are administered a sufficient time before challenge and provided that the challenge dose of antigen employed is not too high.

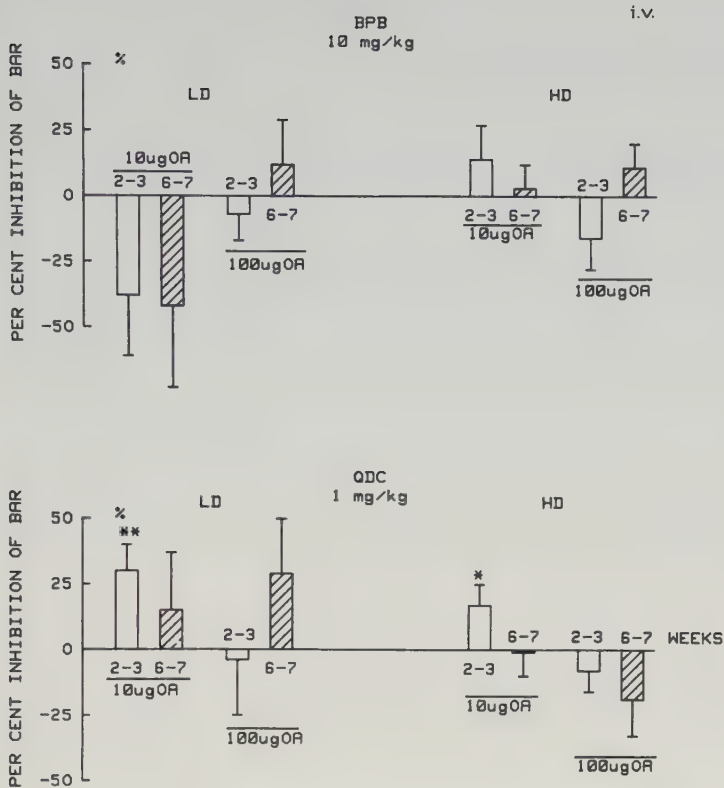


FIGURE 7

Effect on BAR of treatment with BPB or QDC

Rats were immunized with 10 μ g or 100 μ g OA and alum and were used for tests 2-3 or 6-7 weeks after sensitization as indicated. Challenge was performed as described in Material and Methods. Pretreatment was performed 5 min before challenge, with BPB (10 mg/kg i.v.) or QDC (1 mg/kg i.v.) as indicated. Results are shown as percent inhibition of response recorded in vehicle-treated animals. Each figure is based on at least 3 experiments, each consisting of at least 4 drug-treated and a similar number of vehicle-treated animals. Statistics as in legend to Figure 1.

The inhibitory effects of GCS examined are dose-dependent

These results conform to the findings of Church and coworkers (6,8) who recorded a dose-related inhibition by DEX of BAR in rats sensitized by infection with *Nippostrongylus brasiliensis*. Peak activity was recorded by these authors 12-24 hours after

oral administration and the approximate ED_{50} value was found to be 1.8 mg/kg (oral) and approximately 0.4 mg/kg (i.p. administration). In our hands, the examined GCS are at best only slightly more effective after i.t. instillation than after being given by the i.p. route. On the other hand, Stotland and Share (38) reported that DEX (1 or 10 mg/kg) given orally 18 h before challenge did not significantly alter the antigen-induced bronchial response. In their experiments the rats were sensitized with OA in alum and supplementally injected with B. pertussis vaccine. These authors challenged the rats with a maximum dose of antigen (10 mg/kg). In our experiments, too, such a high challenge dose of antigen elicits an airway response which at best can be marginally reduced by GCS treatment. However, methysergide and 1-benzylimidazole, can effectively reduce the BAR elicited by such a high challenge dose of OA (13), which shows that such BAR can be reduced by drug treatment.

The GCS do not inhibit BAR completely

These results also conform to previous findings in antigen-induced anaphylactic reactions in vivo (1,6,38), that inhibitory effects of GCS are seldom complete. The reason for this is not clarified but since mast cell mediator release is at best only partially blocked by GCS (2,25,32,33,34), and since the spasmogenic effect of the main mediators of anaphylaxis is at best only slightly reduced (7,27) it may be expected, that GCS would be unable to totally block a BAR.

GCS have to be administered a sufficient time (12-24 h) before challenge to exert inhibition

If tests were performed 5 hrs after its i.p. administration, BUD could even significantly increase the BAR. Similarly, Church & Miller (8) found pinna anaphylaxis in mice to be potentiated by DEX given 1-2 h before challenge, and in the hamster cheek pouch model, vascular permeability is increased by BUD if the drug is given 15-30 min before test but reduced if administration of the drug precedes test by 60 min (Svensjö, E. pers. commun.). In the sheep model, methyl prednisolone succinate (15 mg/kg) completely blocked antigen-induced airway reactions if given 3 h before

(but not 20 min before) challenge (14). GCS given at the latter time point did not aggravate the reaction. The reasons for the potentiating effects of GCS seen in some models including the present are not clear.

Inhibitory effects of GCS do not vary with immunization conditions

In the guinea pig model, Andersson & Brattsand (1) demonstrated a partial inhibitory effect of BUD on a BAR presumably mediated by IgE-antibody but not on a BAR mediated by IgG. In our rat model, a significant correlation between BAR, induced by a low antigen challenge dose, and serum OA-IgE levels was recorded in animals immunized with 10 ug OA 14 days (but not 42 days) before test (12). Similarly, BAR induced by high allergen challenge doses correlated significantly with serum levels of OA-IgE, but not with OA-IgG_{2a}, in animals immunized with 10 ug or 100 ug OA 42 days before test but not at the earlier time point. There is no indication in the present data that inhibitory effects of GCS were more pronounced under conditions where BAR correlated to serum OA-IgE levels.

Mechanism of action of GCS and GCS effects are not mimicked by putative phospholipase A₂ inhibitors

The mechanism of action of the GCS on rat BAR is not unravelled by the present experiments. From previous work one can conclude that mediator-antagonism may play at best a minor role (7). Rather, one would suspect that the GCS inhibits antigen-induced mediator release. The time dependency of the effect would fit such a mode of action (2,25,32, 33) and the 'synergistic' effect recorded by Stotland and Share (38) and by us (Figure 6) with combinations of DEX or BUD and DSCG would also suggest that DSCG and GCS both act (at least in part) by an 'anti-allergic' effect mechanism or that GCS reduces increased permeability on the endothelial cell level (3).

On the biochemical level GCS are thought to inhibit mediator release by inducing synthesis/release by, e.g., PMNs of lipomodulin/macroscortin, which by virtue of its phospholipase A₂-inhibitory properties attenuates the stimuli-induced release of

arachidonic acid from phospholipids (4,19). Two other compounds, which have been attributed phospholipase A_2 -inhibitory potential, are BPB and QDC. We are aware of that these compounds show little specificity for phospholipase A_2 and results obtained with these drugs should be interpreted with caution (17,20,22). However, BPB and QDC are potent and effective inhibitors of immunologically induced histamine release from human basophils and lung mast cells (for ref. see 3). In the present study BPB does not markedly affect the BAR even after a dose of 10 mg/kg irrespective of the immunization condition of the test animals and QDC only marginally reduces the BAR (Figure 7). Moreover, the effect pattern of QDC does not conform to that of the GCS (Figure 1). Therefore, the results obtained with BPB and QDC lend little support to the possibility that inhibition of phospholipase A_2 -activity would lead to inhibition of antigen-induced BAR.

Possible effect mechanisms for the GCS other than phospholipase A_2 -inhibition include induction of (i) an increase in rat mast cell or possibly respiratory smooth muscle β -receptor number (24,25), (ii) the GTP-dependent regulatory component of the adenylate cyclase (21), or (iii) the cAMP-dependent protein kinase activity (23). Higgs and coworkers (18) recently showed that BW 755C (3-amino-1- m-(trifluoromethyl)phenyl -2-pyrazoline), an inhibitor of both the cyclooxygenase and lipooxygenase pathways of arachidonic acid metabolism, in a carrageenin-polyester sponge-induced model of tissue damage mimicked the effect of DEX. We have previously shown that the effect of BW 755C in the rat BAR model varies with immunization conditions (13). On the other hand, indomethacin is a partial inhibitor of BAR even when elicited by a high antigen dose (13), which is not the case with DEX. Although doses and timing of administration of the drugs differed in these experiments, one would suggest that GCS inhibit BAR by virtue not only of its blocking the stimulus-induced generation of arachidonic acid metabolites but other mechanism(s) of action also contribute to the effect.

In the present model, 0.1 mg/kg of BUD given i.p. a sufficient time before challenge marginally reduced a BAR elicited by a low antigen challenge dose. In asthmatics, daily doses of prednisone

averaging approximately 17 mg in one study (36) and ranging from 20 to 250 mg in another (29) relieved asthma symptoms but transiently increased serum levels of specific and total IgE. We found no effects of a three-week treatment with BUD (0.1 mg/kg i.p.) on BAR or on serum levels of specific or total IgE when assessed one week after end of treatment.

In conclusion, GCS reduce bronchial anaphylactic reactions induced in actively sensitized rats by a low antigen challenge dose but only marginally those induced by a high challenge dose of allergen. Contrary to what was previously found with some other drugs, the effect of the GCS is not markedly dependent on immunization conditions of examined animals. However, the GCS has to be given at least 5 h, independent of route of administration, before test to mediate inhibition.

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VI

VI

ANTIGEN-INDUCED BRONCHIAL ANAPHYLAXIS IN ACTIVELY
SENSITIZED SD RATS: EFFECTS OF LOCAL TREATMENT
WITH ANTI-ASTHMATIC DRUGS.

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Key words: aerosol, bronchial anaphylaxis, budesonide, DSCG,
terbutaline, theophylline, rats.

Abbreviations used:

BAR	Bronchial anaphylactic reaction
BUD	Budesonide
DEX	Dexamethasone
DSCG	Disodium cromoglycate
GCS	Glucocorticosteroid(s)
HD	High dose of antigen challenge
i.p.	Intraperitoneal(ly)
i.t.	Intratracheal(ly)
i.v.	Intravenous(ly)
LD	Low dose of antigen challenge
OA	Ovalbumin
SD	Sprague Dawley
TERB	Terbutaline
THEO	Theophylline

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SUMMARY

We studied the effects of anti-asthmatic and anti-inflammatory drugs on antigen-induced bronchial anaphylactic reactions (BAR) in SD rats immunized with ovalbumin (OA) and alum. The animals were treated locally (intratracheal instillation (i.t.) or by aerosol) with terbutaline (TERB), disodium cromoglycate (DSCG), theophylline (THEO), the xanthine derivative 3,7-dihydro-1,8-dimethyl-3-phenyl-1H-purine-2,6-dione (D4026), budesonide (BUD) or dexamethasone (DEX) at various times before intravenous (i.v.) challenge with OA. The BAR was elicited by giving a low dose of OA. When the response to that challenge had levelled an additional high dose of antigen was given.

Previous work had shown that TERB, DSCG, and D4026 given systemically (i.v.) at a suboptimal dose, had a better inhibitory efficacy when the animals were challenged with a low antigen dose late (6 weeks) than when challenged early (2-3 weeks) after immunization. We show here that such a difference in efficacy is not recorded after local treatment. Moreover, each of the drugs inhibited BAR to the same extent after i.t. as after i.v. treatment. Potent drugs like TERB and D4026 seemed to show similar efficacy when given i.t. as when administered by aerosol, whereas less potent drugs like DSCG and THEO were less effective when given by aerosol.

In a previous study, we showed that the inhibitory capacity of glucocorticoids (GCS) on the BAR did not vary with sensitization conditions of the rats. BUD and DEX showed the same inhibitory capacity after intraperitoneal (i.p.) treatment as after i.t. treatment. In the present study, BUD and DEX showed the same inhibitory capacity when given by aerosol as compared with i.t. or i.p. administration. The duration of the anti-anaphylactic activity after inhalation was longer for DEX than for BUD.

Clinical aspects

Suboptimal doses of anti-asthmatic drugs such as TERB, DSCG, THEO and D4026 given systemically (i.v.) have previously been shown to better express inhibitory effects on immediate bron-

chial anaphylaxis in rats (BAR) when the animals were challenged with a low antigen dose at 6 weeks than when challenged at 2-3 weeks after immunization. We showed that with local treatment (i.t.) no such difference was recorded. Moreover, TERB and D4026 seem to be as effective by aerosol as when given i.v. or i.t., whereas DSCG and THEO are not. BUD and DEX showed the same inhibitory capacity when given by aerosol as when given i.t. or i.p. These data show that the route of administration may influence the anti-anaphylactic efficacy of drugs.

INTRODUCTION

Inhalation is by many authors considered a favourable route of drug administration in asthmatics. In this way one may gain efficacy at lower therapeutic doses, with a faster onset of action, with longer duration and finally with less side effects than after, e.g., oral treatment (1,8,9,10,20,24). For example, inhaled glucocorticoids (GCS) such as budesonide (BUD) are highly effective in the treatment of chronic asthma apparently without inducing marked side effects (9,23,33).

Moreover, local treatment may enhance favourable drug effects. For example, patients with asthma may suffer from retarded cilia beat frequency. Inhaled β_2 -agonists and GCS increase the mucociliary clearance rate and improve the degree of expectoration in these patients (18,25). The prophylactic anti-asthmatic drug disodium cromoglycate (DSCG) is in man effective only when taken by the inhaled route (6). On the other hand methylxanthines (e.g. theophylline (THEO)) are usually given either orally or, in the treatment of severe cases of asthma, by i.v. administration. When given as an aerosol, xanthine derivatives induce a low, if any, bronchodilation in asthmatic patients compared with that induced by β_2 -agonists (2,11,21,31).

We have previously characterized in some detail the antigen-induced immediate bronchial anaphylactic reaction (BAR) in Sprague Dawley (SD) rats with respect to drug effects employing candidates from the above mentioned groups (12,14). We found

that, given i.v., TERB, DSCG and the xanthine derivative D4026 more effectively inhibited the BAR when rats were challenged at 6 weeks after immunization than they did at 2-3 weeks. However, the effects of GCS did not differ in this way with the time of challenge. In the present study, we examined the effects of these drugs when given locally (either i.t. or by aerosol) to compare their efficacies with those observed following systemic administration (i.v. or i.p.).

MATERIALS AND METHODS

Animals and immunization. Outbred male Sprague Dawley (SD) rats of SPF status (Møllegaard Breeding Center Ltd, Ejby, Skensved, Denmark) were used at a weight of 200 g. After arrival at our laboratory the animals were kept under conventional housing conditions. They were immunized by an i.p. injection of 10 μ g ovalbumin (OA; Sigma grade III, crystallized and lyophilized) in 0.5 ml saline mixed with 100 mg dried and resuspended $\text{Al}(\text{OH})_3$ (F2200, Reheis Chemical Company; obtained through AB Astra, Södertälje).

Drug administration. Drugs were administered either i.v., i.t. or by aerosol at the indicated time after sensitization. When drugs were given by i.t. instillation, the animals were lightly anaesthetized with ether and placed on a slanted board. A five-centimetre-long cannula was inserted down to a point just above the bifurcation of the trachea. Occasionally, the trachea was opened to ensure a proper position of the needle. After the drug was delivered, the rats remained in a leaning position until they woke up.

Conscious animals were exposed to an aerosol that was generated by a medical nebulizer MA2 (Viasol, Malmö, Sweden) at a flow rate of 4.5 l/min and at an air pressure of 0.5 MPa. Generated particles from a 0.9% NaCl solution were measured by an Electrical aerosol analyzer (EAA, TSI 3020). The mass mean aerodynamic diameter (MMAD) of dry particles was 0.51 μm , with a geometric standard deviation ($\tilde{\sigma}_g$) of 2.0 from either a water

based solution or an alcohol solution. For further output characteristics see Dahlbäck (15). The aerosol was then led into a flow past inhalation chamber (Battelle, Geneva), where the animals were exposed nose-only for 10 or 15 minutes (7). The aerosol generated from a defined concentration of each drug was withdrawn (0.2 l/min) on filters for determination of the dose. DSCG was extracted by a phosphate buffer pH 7.4 and determined spectrophotometrically at 326 nm, while the other drugs were measured by the use of high performance liquid chromatography at our analytical laboratory. U. Espmarker, U. Linnarsund, T. Löf and P. Tiensuu are gratefully acknowledged.

Respiratory measurements. At the indicated time after immunization, the animals were used for test. They were anaesthetized with 40 mg/kg pentobarbital (Mebumal^R, ACO, Sweden) and 25 mg/kg metomidate chloride (Hypnodil^R, LEO, Sweden) given i.p., 5 mg/kg succinylcholine (Celocurin^R, Vitrum, Sweden) was given i.p. in order to prevent spontaneous respiration. Respiratory measurements were performed according to the method of Stotland & Share (32) slightly modified as described by Dahlbäck (12). In brief, the trachea was cannulated and the rat ventilated with a Brown constant volume respiratory pump in a closed system. Intratracheal pressure was recorded through a side arm of the cannula connected to a Statham P23AC pressure transducer. The respiratory rate was set at 70 strokes/min with a tracheal pressure of 8 cm H₂O (tidal volume range 2.0 - 3.75 ml). All recordings were made on a Grass polygraph model 7B. The animals were challenged through a catheter inserted into the right jugular vein.

Antigen challenge. Unless otherwise specified, challenge was performed 30 min after i.t. or aerosol administration of drugs or 2 min after i.v. injection of drugs. A low antigen dose (LD; 0.3 mg OA) was first injected and the response was recorded. Approximately 2 min later (when the response to the low antigen dose had levelled) an additional high dose of antigen (HD; 5 mg OA) was injected and the response again recorded. The increase in intratracheal pressure resulting from each antigen injection was calculated as per cent of the prechallenge pressure. At the low antigen dose this increase was referred to as $\Delta P\%$, at the high antigen dose it was called $\Delta P_{max}\%$.

Drugs. The sources of the drugs used were Apoteksbolaget, Sweden (disodium cromoglycate, DSCG, Lomudal), AB Draco, Sweden (terbutaline inhalation solution, TERB; budesonide, BUD; 3,7-dihydro-1,8-dimethyl-3-phenyl-1H-purine-2,6-dione, D4026), Knoll, West Germany (theophylline, THEO), Lark, S.P.A., Milano, Italy (dexamethasone, DEX). For i.v., i.t. and aerosol administration, DSCG (dissolved in saline) and terbutaline inhalation solution were used. The xanthine derivatives were dissolved in equimolar amounts of 0.5 M NaOH/saline and further diluted in saline. Budesonide and dexamethasone were dissolved in 95% ethyl alcohol. The volume of each drug administered i.t. was 0.05 ml/100 g body weight. None of the vehicles influenced the response to antigen.

The following formula has been used for approximation of lung burden of each animal.

$$\frac{CC \times RMV \times ET \times D}{BW} \quad \text{mg/kg}$$

CC, chamber concentration; RMV, respiratory minute volume = $2.1 \times (\text{wt. in grams})^{0.75}$ ml (19); ET, exposure time; D, per cent of inhaled aerosol; BW, body weight. The figure used for D is 20% (30).

In each experiment a similar number of vehicle- and drug-treated animals from the same sensitization pool were tested. Mean values of $\Delta P\%$ and $\Delta P_{\text{max}}\%$ were calculated for each group. Per cent inhibition of the anaphylactic reaction was calculated as follows:

$$\% \text{ inhibition of BAR} = 100 \times \left(1 - \frac{\text{Mean } \Delta P\% \text{ or } \Delta P_{\text{max}}\% \text{ of drug-treated animals}}{\text{Mean } \Delta P\% \text{ or } \Delta P_{\text{max}}\% \text{ of vehicle-treated animals}} \right)$$

Figures given in graphical illustration are mean values $\pm$ S.E. for % inhibition of BAR calculated from experiments each of which included at least five drug-treated and five vehicle-treated animals. Significance of inhibition was assessed by analysis of variance with linear contrasts on vehicle- and drug-treated animals.

RESULTS

We have previously examined the inhibitory effects on the BAR of i.v. administered TERB, DSCG, THEO and D4026 (12) as well as those of BUD and DEX (14). Since THEO was ineffective at the dose then examined (0.3 mg/kg), a dose-response study of that drug was now performed. THEO was given i.v. at a dose ranging from 0.1 to 25 mg/kg to animals immunized with 10 μ g OA and challenged either early (2-3 weeks) or late (6 weeks) after immunization. As shown in Fig. 1 THEO at the dose level 1 mg/kg is significantly ($P<0.05$) more effective in animals challenged with a low antigen dose 6 weeks (LD) after sensitization than in those examined earlier. Note, that THEO inhibits the BAR by 50-65% at a dose level of 25 mg/kg independent of challenge dose and time of challenge. Note also, that THEO is more potent and slightly more effective in animals challenged early than in those challenged late when a high challenge dose of antigen is employed.

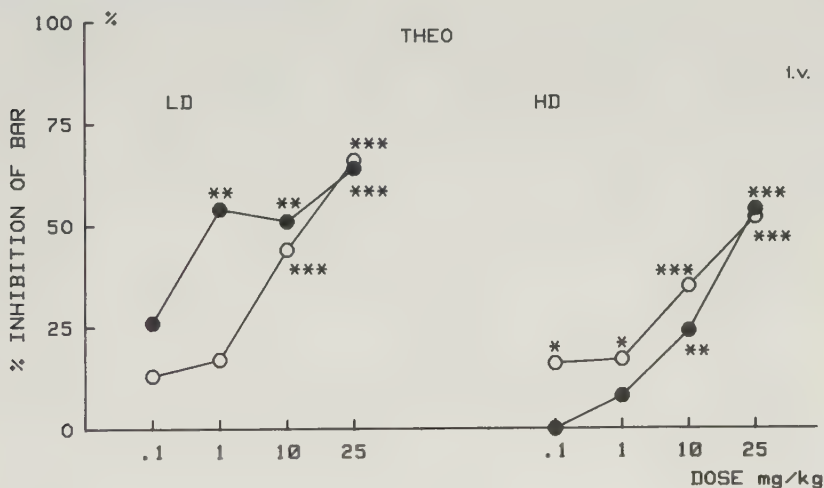


FIGURE 1

Effects on BAR of i.v. administered THEO

THEO was given i.v. to immunized rats (10 μ g OA and alum) either 2-3 weeks (open circles) or 6 weeks (closed circles) after immunization. Challenge was performed with a low dose (LD; 0.3 mg OA) and a high dose (HD; 5 mg OA) of antigen given 2 min after drug delivery. The effects of THEO are shown as per cent inhibition of response recorded in drug-treated vs vehicle-treated animals (see Materials and Methods).

* $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Effects on the BAR of anti-asthmatic drugs administered by i.t. instillation

TERB, DSCG, THEO and D4026 were administered to immunized (10 μ g OA) animals by i.t. instillation 30 min before challenge. The animals were examined at either 2-3 weeks or at 6 weeks after immunization. The results show (see Fig. 2) that all drugs except THEO inhibited the BAR in a dose-related fashion when the animals were challenged with a low provocation dose (0.3 mg OA) either at 2-3 weeks or at 6 weeks after immunization. TERB and D4026 were the most potent of the drugs. They both exhibited an almost linear response relationship between 0.1 and 100 μ g/kg which did not markedly vary with the time for challenge after immunization. The potencies of DSCG and THEO were lower than those of TERB and D4026. The efficacy of DSCG tended to be higher in animals examined 6 weeks after immunization than in those examined earlier. However, this difference did not reach statistical significance. At the high challenge dose of OA, the degrees of inhibition obtained even at the highest examined doses of the drugs were low except for D4026 which showed a 45% inhibition of the BAR. Note also the flat dose response curves recorded for inhibition of the BAR by TERB and by THEO in animals challenged 6 or 2-3 weeks after immunization, respectively, with the high or the low dose of antigen, respectively.

Effects on BAR of anti-asthmatic drugs delivered by aerosol

The effects of TERB, DSCG, THEO and D4026 delivered to immunized animals 30 minutes before a challenge 6 weeks after their immunization are shown in Fig. 3. DSCG and THEO were delivered from solutions nebulized from the highest possible concentration (50 mg/ml) and from 5 mg/ml. TERB and D4026 were nebulized from solutions at doses ranging from 0.05 to 5 mg/ml. The aerosol concentration and the calculated delivered dose (lung burden, μ g/kg) of each drug are presented in Table 1. The results (Fig. 3) show that TERB and D4026 effectively reduce the BAR induced by a low antigen dose, that DSCG is virtually ineffective, and that THEO apparently is only partially effective. No convincing protection was seen for any of the drugs at the high provocation dose except for D4026 at 5 mg/ml.

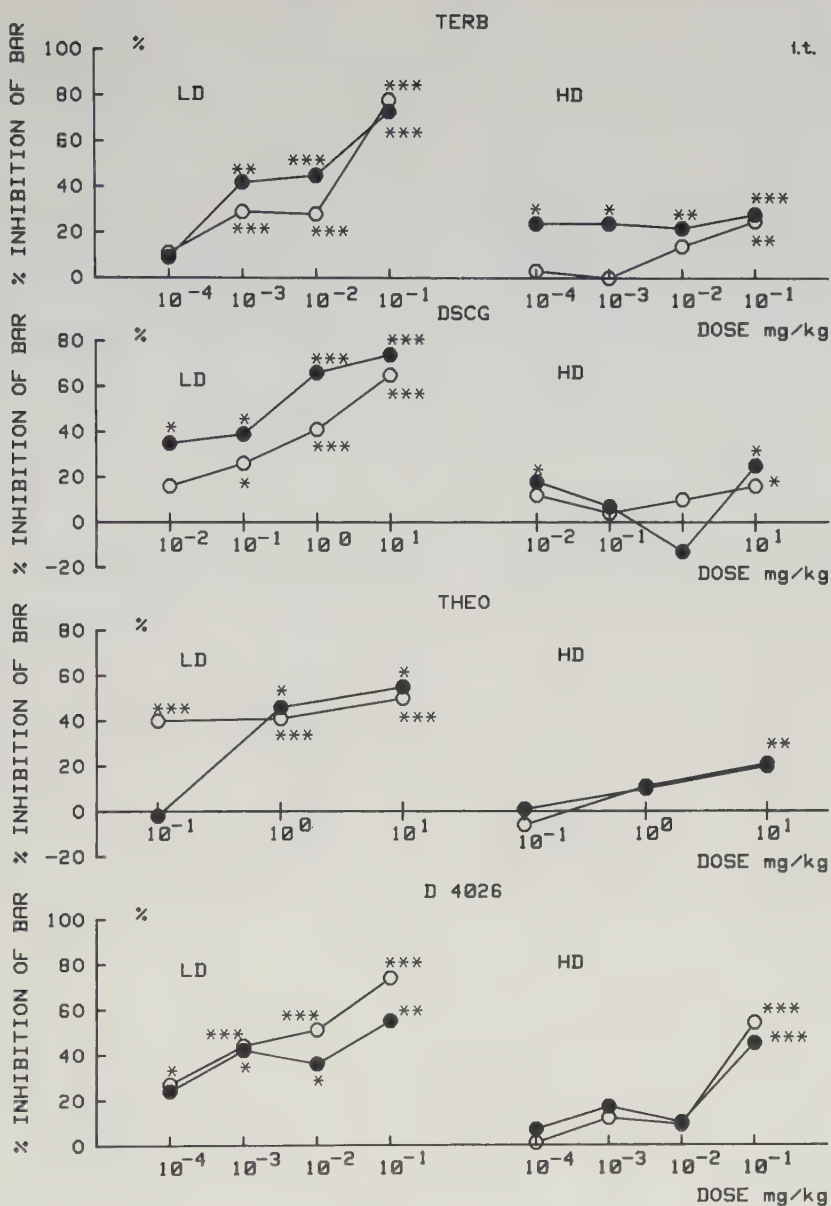


FIGURE 2

Effects on BAR of i.t. administered TERB, DSCG, THEO and D4026

Rats immunized with 10 μ g OA and alum were given TERB, DSCG, THEO and D4026 by i.t. instillation either 2-3 weeks (open circles) or 6 weeks (closed circles) after immunization. Challenge was performed as described in Figure 1 30 min after drug delivery. Effects are shown as per cent inhibition of BAR in drug-treated vs vehicle-treated animals (see Materials and Methods).
 * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

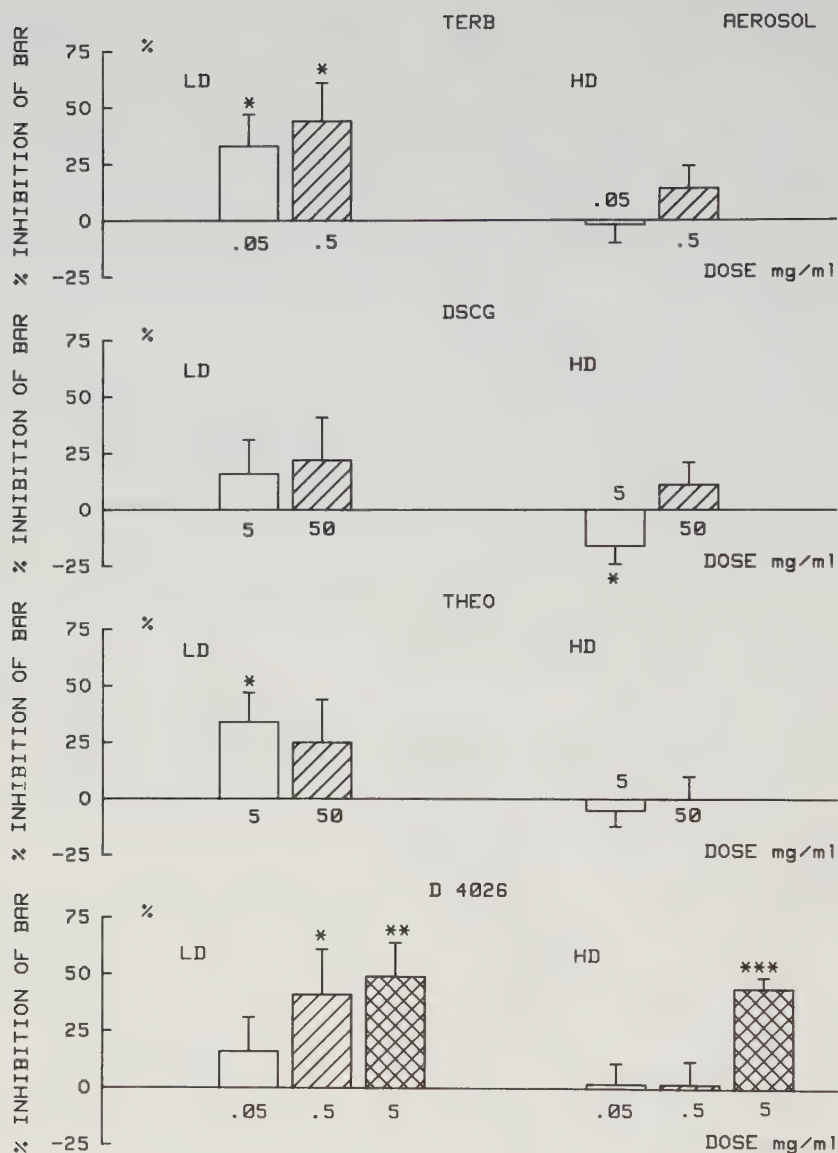


FIGURE 3

Effects on BAR of aerosol administered TERB, DSCG, THEO and D4026

Rats immunized with 10 μ g OA and alum were given TERB, DSCG, THEO or D4026 by aerosol 30 min before challenge, 6 weeks after immunization. Challenge was performed as described in Figure 1. Each column denotes the mean percentage inhibition of BAR of drug-treated vs vehicle-treated animals and SE at each dose level (see Materials and Methods).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

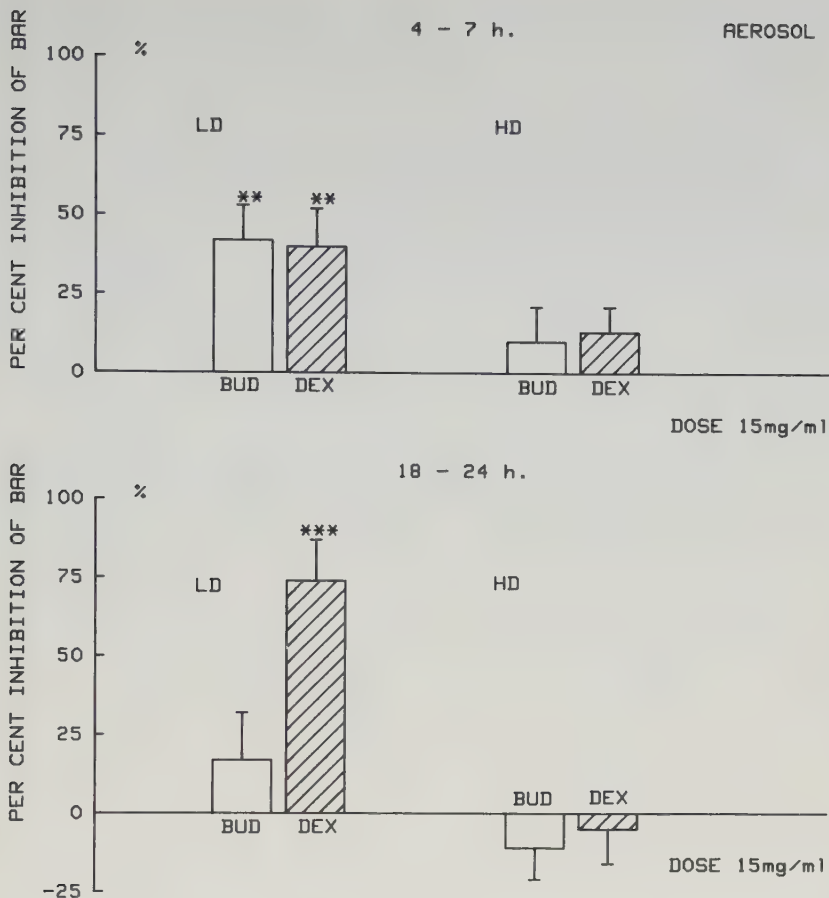


FIGURE 4

Effects on BAR of aerosol administered BUD and DEX

Rats immunized with 10 μ g OA and alum were given BUD (open bars) or DEX (hatched bars) as an aerosol and challenged 4-7 h or 18-24 h after treatment with a low dose (LD; 0.3 mg OA) and a high dose (HD; 5 mg OA) of antigen at 2-3 weeks after immunization. Each column denotes the mean percentage inhibition of BAR of drug-treated vs vehicle-treated animals and SE (see Materials and Methods).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Effects on BAR of selected GCSs given by aerosol

Since the effects on BAR of i.p. administered GCS were previously shown to be independent of immunization conditions (14), animals immunized 2-3 weeks before the experiments were employed

for tests of GCS. The animals were exposed to an aerosol of either BUD or DEX (15 mg/ml). Challenge was performed 4-7 h or 18-24 h after treatment. The results show (Fig. 4, Table 1) that BUD and DEX at the low challenge dose of OA exhibited a similar degree of inhibitory capacity (40%) 4-7 h after administration. DEX but not BUD demonstrated a significantly ($P<0.05$) increased inhibitory capacity at 18-24 h. No inhibitory effect was seen at the high challenge dose on any occasion.

Table 1

Aerosol concentration, estimated lung burden and effects on BAR

Drug	solution ($\mu\text{g/ml}$)	Aerosol concentration ($\mu\text{g/l air}$)	Estimated lung burden $\mu\text{g/kg}$	% Inhibition of BAR ⁺	
				LD	HD
TERB	500	5 $\pm$ 0.5	4	44 $\pm$ 17*	14 $\pm$ 10
	50	NM	(0.4) ^o	33 $\pm$ 14*	-2 $\pm$ 8
DSCG	50 000	710 $\pm$ 50	700	22 $\pm$ 19	11 $\pm$ 10
	5 000	76 $\pm$ 6	70	16 $\pm$ 15	-16 $\pm$ 8
THEO	50 000	510 $\pm$ 20	500	25 $\pm$ 19	0 $\pm$ 10
	5 000	73 $\pm$ 3	70	34 $\pm$ 13*	-5 $\pm$ 7
D4026	5 000	67 $\pm$ 3	60	49 $\pm$ 15**	44 $\pm$ 5***
	500	6 $\pm$ 0.5	6	41 $\pm$ 20*	2 $\pm$ 10
	50	NM	(0.6) ^o	16 $\pm$ 15	2 $\pm$ 9
BUD	15 000	1005	950	42 $\pm$ 11 ^a	10 $\pm$ 11
				17 $\pm$ 15 ^b	-11 $\pm$ 10
DEX	15 000	1363 $\pm$ 21	1300	40 $\pm$ 12	13 $\pm$ 8
				74 $\pm$ 13	-5 $\pm$ 11

NM = not measured; + see Materials & Methods; o extrapolated dose; ^a 4-7 h; ^b 18-24 h;

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

DISCUSSION

A local delivery of drugs which exert their primary therapeutic effect within restricted regions of the body leads theoretically to several advantages over systemic therapy. Of the anti-asth-

matic drugs used in this experimental study, TERB, DSCG, BUD and DEX are all thought to act primarily within the airways and lung and they have all demonstrated valuable anti-asthmatic efficacy in man when delivered by inhalation. Such a primary site of action for THEO may be questioned since the efficacy of inhaled THEO in human studies is not impressive (2,11,21,31). D4026 has not been used in man owing to its toxicity in rats (13). In animal studies it is a bronchodilator in vitro (Dahlbäck, Ekman, Erjefält and Persson, unpublished observation) supporting a primary action for it within airways.

In the present study, two methods were used for "local" drug delivery to the airways and lung, i.t. instillation and inhalation by nose only exposure. Experiments with dusts have demonstrated that inhalation and i.t. instillation result in partly different distributions of particles within the lung (3,26). I.t. instillation has several advantages over aerosol exposure: (i) an exact amount of the drug can be instilled, (ii) by modifying the i.t. instillation technique it is possible to deliver material into one lobe and to have other lung lobes as controls (16), (iii) the consumption of the drug is less compared with that of aerosol generation. A disadvantage of i.t. instillation is, owing to the actual instillation technique and the posture of the animal at instillation, the possibility of an uneven distribution of the drug between different lobes. With aerosol administration the delivered particles are more evenly deposited within the lung, but the amount of drug delivered can be only roughly estimated (see Materials and Methods). In nose breathing animals the size of inhaled particles should be $<1\text{ }\mu\text{m}$ in diameter (27,29) to get most of the particles deposited in the lung.

In the present experimental set-up in rats, the examined drugs when inhaled or instilled into airways all demonstrated anti-anaphylactic properties provided that the challenge was performed with the low antigen dose. The only drug inhaled that did not inhibit BAR was DSCG. Only D4026 showed a marked protection against the high antigen dose. Contrary to the situation seen with i.v. administration (12), the efficacy of the drugs was as good when they were examined after 2-3 weeks as it was 6 weeks

after immunization of the animals.

TERB showed a significant anti-anaphylactic activity in doses $\geq$ 0.4 $\mu\text{g/kg}$ when given by i.t. instillation or by inhalation. When dose-response relationships were compared with earlier studies employing the i.v. route of administration (12), and the required doses to inhibit the BAR by $\sim 60\%$ were compared, the same potency for the compound was found after i.v. injection as after i.t. instillation (Fig. 5). When i.t. instillation and aerosol administration were compared at $\sim 45\%$ inhibition of the BAR (only low aerosol doses were tested) the required doses were again found to be similar (Fig. 5 and calculated doses in table 1).

For D-4026 the threshold dose for significant anti-anaphylactic activity was estimated to be 1 $\mu\text{g/kg}$ for i.t. instillation and 6 $\mu\text{g/kg}$ for inhalation (Figs. 2, 3 and Table 1). To inhibit the BAR-response by $\sim 50-75\%$, higher doses were needed ($\sim 60-100$ $\mu\text{g/kg}$) and at this level of inhibition similar potencies were demonstrated for inhalation, i.t. instillation and i.v. injection (Fig. 5).

In an earlier study of effects on BAR following i.p. injection or i.t. instillation, we found for BUD and DEX that doses of 1 mg/kg were needed to demonstrate significant anti-anaphylactic activity (14). However, when BUD and DEX were compared at 18-24 h with different routes of administration (Fig. 6), it was obvious that the i.p. and i.t. routes had a similar efficacy. When ~ 1 mg/kg of GCS was delivered by inhalation (Fig. 4) significant protection was demonstrated for both BUD and DEX when given 4-7 h before challenge. Only DEX effectively reduced the BAR when given 18-24 h before delivery. The difference between the two drugs at 18-24 h which was also seen when they were given i.t. (14) may depend on the shorter half life of BUD (28) than of DEX (17), and may also depend on the fact that there is a very fast absorption and high bioavailability of BUD deposited in the lung (4,22). With the low doses used in the aerosol experiments this difference in half life probably greatly influences the duration of action of the two drugs.

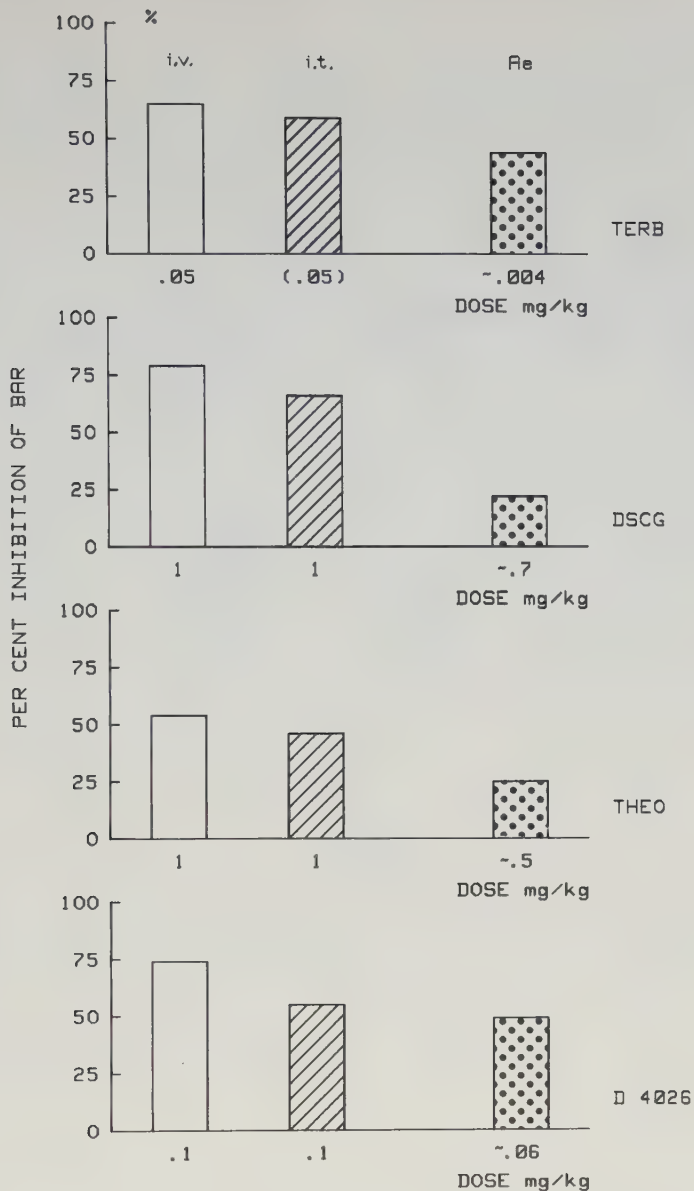


FIGURE 5

Effects of routes of administration on inhibition of
BAR by TERB, DSCG, THEO, D4026

Immunized rats were given TERB, DSCG, THEO and D4026 either i.v. (open bars), i.t. (hatched bars) or by aerosol (Ae, dotted bars), both the latter two were administered 30 minutes before challenge with an LD. Figures in brackets denote extrapolated dose. Effects on BAR after i.v. administered doses of TERB, DSCG and D4026 are taken from (13).

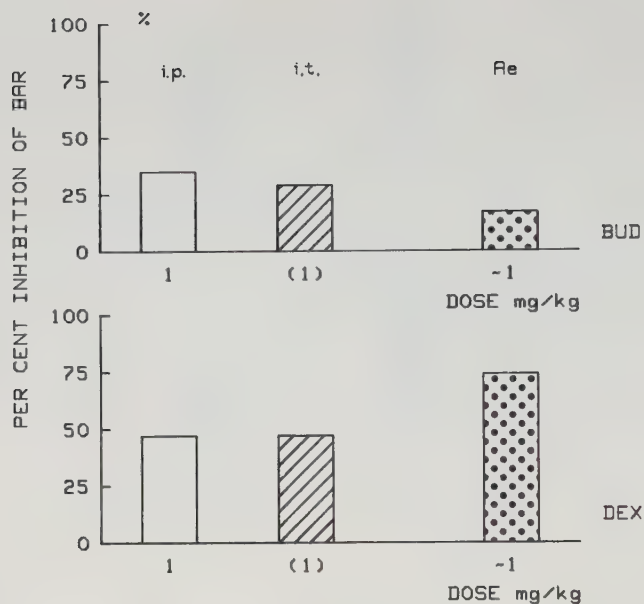


FIGURE 6

Effects on BAR of different routes of administered GCS

Immunized rats were given BUD and DEX either i.v. (open bars), i.t. (hatched bars) or by aerosol (Ae, dotted bars) 18-24 h before challenge with an LD. Figures in brackets denote extrapolated dose. Effects on BAR after i.p. or i.t. administered drugs are taken from (15).

The threshold dose for THEO to block the BAR 6 weeks after immunization was 1 mg/kg after both i.v. and i.t. administration. It was not possible to clearly judge THEO's activity when it was given by inhalation since the highest possible test dose attainable was $\sim 500 \mu\text{g/kg}$. Not surprisingly this dose lacked significant protective activity.

DSCG demonstrated significant anti-anaphylactic activity after i.t. instillation at $100 \mu\text{g/kg}$. When the doses required to block the BAR by $\sim 70\%$ were compared for the i.v. and the i.t. routes,

a similar potency was obtained (Fig. 5). Surprisingly, it was not possible to demonstrate anti-anaphylactic activity for inhaled DSCG despite the fact that the highest dose used was estimated to be ~ 700 $\mu\text{g/kg}$ (i.e. 7 times higher than the threshold dose for recorded activity after i.t. administration). As DSCG was active i.t. but not via aerosol it ought to be clarified if this substance was relatively more retained in the upper airways than what may have been the case for the other substances.

In conclusion, in the rat model, we have demonstrated the anti-anaphylactic activity of TERB, DSCG, THEO, D-4026, BUD, and DEX after i.t. instillation. However, none of these drugs showed a higher potency or efficacy after this "local" mode of administration than they did after i.v. or i.p. injection. This is in accordance with results obtained in a Sephadex-induced rat model of lung oedema (5), where BUD showed a similar potency irrespective of whether it was given by i.v. injection, i.t. instillation or by inhalation. Thus, more detailed studies are required to find out whether it may be possible to better define the extent of local activity of a particular drug in the airways and also to speculate about the possibility of developing new drugs with more selective local activity by inhalation.

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There was a young rat from pharmacology
who had studied some particle technology
"If this aerosol I take
then my bronchi dilate,
but, just what about my lung physiology?"

